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**REPORT OF THE  
NORTHERN REGIONAL RESEARCH CENTER  
DECEMBER 31, 1978**

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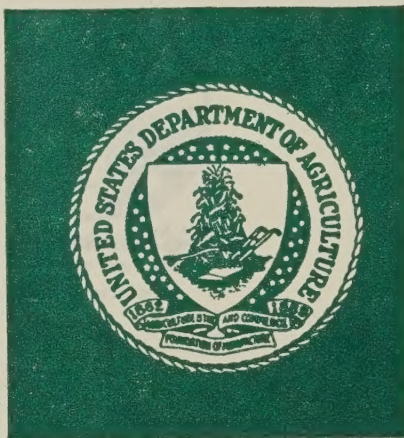
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PROGRESS REPORT  
OF THE  
NORTHERN REGIONAL RESEARCH CENTER  
December 31, 1978

INTRODUCTION

The Northern Regional Research Center, located at Peoria, Illinois, is one of the major research centers of the North Central Region, Agricultural Research, Science and Education Administration (SEA-AR), U.S. Department of Agriculture. Basic and applied research is conducted in the physical and biological sciences and in engineering. Northern Center scientists cooperate with representatives of colleges and universities, State experiment stations, research institutes and associations, industrial organizations, and with other Government agencies. Much of the cooperation is informal, but some work is conducted under cooperative agreements and memorandums of understanding. In addition, the Center's program is supplemented by a variety of research projects in foreign countries under Public Law 480 grants.

Providing scientific information for improvements in the post-harvest sector of American agriculture is a major mission of NRRC. Such improvements in the handling, storing, processing, and distribution technology through which agricultural products move "from farm gate to shopping basket" inevitably benefit both consumers and farmers. For example, research on this complex technology provides the basis for preserving and increasing food quantity, economy, quality, safety, and nutritive value. This research also offers our best hope for substantially reducing the energy intensiveness of post-harvest operations, which collectively require at least twice as much energy as is consumed on farms, and for innovations contributing to maintenance of environmental quality. Responding to these needs and opportunities, NRRC's research program and scientific staff provide SEA-AR's principal effort and expertise in processing and utilizing soybeans, cereal feed grains, and special crops. In addition, the Center is devoting attention to alternative energy resources from agriculture.

Currently most of the soybean research is directed toward decreasing the cost and improving consumer acceptability and nutritional quality of the oil and of high-protein products derived from the meal. This research continues to justify the stature earned by past accomplishments which provided much of the fundamental basis for today's edible soybean oil and food grade soy protein industries. For instance, Center scientists pioneered in development of statistically reliable taste panel procedures that are essential for attaining improved flavor of soy-based foods. In the non-food area a nucleus still exists of a former sizable effort to exploit soybean and other vegetable oils as petrochemical-sparing industrial raw materials. From this nucleus the research could be expended rapidly, should national priorities so mandate.

Cereal grain research, on the other hand, is almost equally concerned with industrial and with feed and food uses. For example, Center scientists' special knowledge of the complex chemical and physical properties of corn



starch and flour has enabled them to develop new technologies with promising on-farm applications. These include water pickup and retention by Super Slurper and controlled release of pesticides through encapsulation with derivatized starch. Recent research in the food area led to corn germ flour, and studies underway on food fiber are aimed at improving the healthfulness of the American diet. In addition, the Center staff continues to be a principal source of information and research on blended foods, such as CSM (from corn, soybean flour, and dry milk solids) for the Food for Peace program. Another major contribution to food science is research to determine the relationship between wheat proteins and their functional properties in bread and other baked goods. Ultimately these studies will provide fundamental insight relevant to nutritional products from other cereals as well as from oilseeds.

A key research resource, the Agricultural Research Culture Collection (NRRL), is a world renowned repository of agriculturally and industrially important microorganisms. Reference cultures, catalogued taxonomic data, and professional expertise associated with this microbial germ-plasm bank have enabled NRRC to make vital contributions and to assume preeminent roles in mycotoxin research and in fermentation technology, including production of food ingredients and fermented foods such as tempeh. Because of the unique capabilities of a multi-disciplinary staff and the importance of the problem, research on mycotoxins has become one of the largest components of the Center's overall effort. This research plays a key role in protecting our food supply from these hazardous substances.

Post-harvest processing behavior and product quality are markedly affected by preharvest factors. Recognizing this, Center chemists, engineers, microbiologists, and physicists participate in joint projects with other SEA-AR and SAES scientists conducting genetic and agronomic studies. Determination of processing and compositional characteristics of plant materials from botanical collections, breeding programs, and studies of soil and atmospheric variables is a major form of such participation. Another involves natural toxicants. Center scientists provide analytical and biochemical information necessary to make sure levels of these minor constituents are not seriously increased in new varieties. This work, like the mycotoxin research, helps assure the safety and nutritional quality of our food supply.

In the newest dimension of the Center's research program its scientists have added their weight to the growing emphasis on photosynthesis, nitrogen fixation, and plant tissue culture. Their novel biochemical, microbiological, and physical approaches complement longer standing studies by plant physiologists and thereby expand and diversify the total SEA-AR attack in these high priority areas.

This report summarizes current research of the Center and lists publications and patents resulting from the research. The research summaries include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are available to those having a special interest in the development of public agricultural research programs.



This report was prepared at the Northern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Peoria, Illinois 61604. Additional copies of the report and reprints of most publications can be obtained from the Northern Regional Research Center. A separate annual listing of publications and patents also is available.

## SELECTED ACCOMPLISHMENTS

### Chemical Derivatization of Cereal Starch by Semi-Dry Reaction Processes.

Cereal derivatives of considerable commercial import, exemplified by cationic ethers of starch and quaternary amine compounds, have been prepared by new synthetic procedures that overcome limitations of classical procedures. Thus, the new methods, through dry blending of reactants, drastically reduce energy requirement/unit by (1) realization of room temperature reactions achieved over several days and (2) elimination of drying requirement or solvent removal and disposal. Additionally, granule swelling-inhibitors are not necessary, thereby providing further cost reduction. The basic process is a radical departure from conventional preparative methods; the driving force results from a 10-20 fold increase of effective reactant concentration yet conditions do lead to homogeneous substitution of reactants. Because of the inherent simplicity of the process and its use of conventional mixing and blending equipment, it is especially valuable to those entrepreneurs with limited resources. The unique reaction process has stimulated plans to initiate fundamental studies of the relatively neglected research area represented by high-concentration derivatizing agents in diffusion-controlled reactions with solid substrates. [See Cereal Products Laboratory (CP). A.1.]

Biodegradable Plastics. Biodegradable plastic mulch, planters, and containers, are urgently needed by farmers to further improve agricultural technology. By studying the interaction between starch and synthetic polymers, ways were discovered for extruding combinations of starch and an ethylene-acrylic acid copolymer that yields films having properties closely resembling those of polyethylene. The films are biodegradable after extended periods of soil exposure, depending upon the composition, and they do not require a water-soluble plasticizer. The study is believed to have overcome major obstacles leading to a feasible biodegradable plastic needed for agricultural applications. Small test samples of the films are being evaluated by USDA scientists in Texas and Florida in connection with programs on automatic transplanter systems for field crops and the field seeding of crops conventionally transplanted. The potential is excellent for extending this starch-in-plastic technology to the 30 billion pounds of plastic now produced annually. The negative impact on the environment that is now evident by the discarding of plastics that do not biodegrade can hopefully be overcome through this technology. (See CP, A.4.)

Controlled Release Pesticides. Our continuing research on the concept of using natural polymers to provide controlled release (CR) of chemicals to improve the efficacy of weed control and reduce the impact of chemicals on nontarget organisms has resulted in successful field tests with selected CR formulations. Cooperating SEA-AR scientists at Beltsville, Purdue University, and the University of Illinois found that starch-encapsulated Treflan CR formulations greatly reduced weed population and extended the period of weed control over standard commercial formulations of this herbicide. The CR formulation also obviated the need for soil incorporation of this herbicide, a requirement with standard formulations due to its volatility and susceptibility to photodecomposition. Tests with other herbicides and soil insecticides also reveal improved efficacy of pest control with starch encapsulated pesticides because losses due to leaching and volatility are greatly reduced. (See CP, A.6.)

Differential Binding of Cellulolytic Enzyme Components. The first step in hydrolysis of cellulose catalyzed by the enzyme complex from the mold Trichoderma reesei produces large soluble polysaccharides called cello-dextrins. The second reaction involves further hydrolysis of these soluble products. It has been presumed that all components of the complex of cellulase enzymes are bound to the cellulose substrate. A suprising discovery is that the enzymes that hydrolyze the cellodextrins are not adsorbed. They are lost when the glucose is withdrawn from a digest. In addition, the enzyme components involved in the initial conversion of cellulose to soluble cellodextrins are adsorbed on the cellulose to a significantly greater extent than theory predicts; this finding suggests the involvement of unidentified mechanisms and factors. Both the loss and excess adsorption of enzyme activity limit the rate and the extent that cellulose is converted to glucose. [See Cereal Science and Foods Laboratory (CSF), A.1.]

New Knowledge on Chemistry of Cooking. Sugars and amines in foods can react to form complex, dark-brown products. Studies of these reactions are fundamental to understanding what happens when food components interact during cooking. In recent years, 6- and 12-carbon reaction products have been isolated and their structures determined. Now, a 22-carbon compound formed in the browning reaction of D-glucose and an amine has been shown to be a complex phenol--more specifically, an aromatic, trihydric, tetramethyl di-indanone. Although a few phenolic compounds previously had been shown to form in trace amounts from D-glucose, they have not been detected before in so high a yield under such mild reaction conditions. Because polymers also form in the same reaction mixtures, scientists conducting these studies theorize that the 12- and 22-carbon compounds polymerize to form the brown polymers that prevail in many cooked foods. (See CSF, A.2.)

Sequence Analysis Reveals Genetic Relationships and Explains Properties of Cereals. Just as a family tree traces a person's ancestry, so is the origin of cereal grains reflected in their proteins as sequences of amino acids. Both wheat and corn contain numerous alcohol-soluble proteins termed prolamines, which are under single gene control. Prolamines of corn, Tripsacum, Teosinte, and sorghum have been shown to have similar amino acid sequences, which indicates the evolutionary interrelationships of these cereals. Examination of amino acid sequences and possible interactions has also contributed to understanding of functional properties of corn and wheat proteins. For example, sequence analysis showed that some wheat prolamines are identical to subunits of glutenin, the protein responsible for dough elasticity. (See CSF, A.3.)

Continuous Procedure for Parching Wild Rice. A continuous procedure for parching wild rice was developed as an alternative for the batch systems now being used by the industry. Quality characteristics of the final product are essentially the same as those of commercially available rice, except that a slightly toasted flavor is lacking. This can be simply added by a slight toast in a continuous roaster or drier. The wild rice industry now has available an alternate procedure for more efficiently processing their product. [See Engineering and Development Laboratory (ED), A.2.]

Control of Microbiological Activity During Low Temperature Corn Drying. A new process for drying grain was developed. Wet grain was dried with low-flow ambient air over a 2- or 3-month period with intermittent applications of



gaseous ammonia to suppress microbial deterioration. The process controlled toxigenic microorganisms, saved energy, and preserved quality. A petition to EPA to allow the use of gaseous ammonia as a mycostatic agent in grain drying was published in the Federal Register in December. (See ED, B.1.)

Unique physiology of an algal nitrogen-fixing partner of a plant. Blue-green algae isolated from Azolla have been found to grow in the dark with an unusual sugar as an energy source. Since only few strains of free-living blue-green algae can grow in this way, the finding suggests that those algae isolated from a nitrogen-fixing partnership with the fern may be less photosynthetic in character than free-living strains. The isolated algae differ from similar free-living strains in that their nitrogen fixation when grown in the dark is two- to threefold higher than in photosynthetically grown cells. The results suggest that, in the fern leaves, the unusual sugar supplied by the host plant provides necessary energy for the algae's growth and nitrogen fixation. [See Fermentation Laboratory (FL), A.1.]

Advances in Microbial Insecticide Production. An alternative organism has been developed for the production of insecticidal protein used in biological control of lepidopteran pest insects. The organism is an asporogenous mutant of Bacillus thuringiensis subsp. kurstaki normally used by industry. Entomocidal preparations made from this organism contain few, if any, spores and therefore minimize the threat of wildlife infection and passage. (See FL, B.1.)

More Accurate Definition of Yeast Species. Accurate identification of yeasts is difficult because species limits are frequently not clearly defined and separations may be based on characteristics that later prove to be of little significance. To overcome this difficulty, microbiologists have used DNA (genetic material) relatedness to separate species. If strains showed 80-100% DNA relatedness, they were considered to belong to the same species while strains showing less than 25% DNA relatedness were considered different species. Values between 25-80% seldom have been found. (See FL, C.4.)

Unusual Starch-Hydrolyzing Lactobacillus Discovered. Until recently all lactobacilli were believed incapable of hydrolyzing starch. Further taxonomic study of 80 lactobacilli isolated from swine waste-corn fermentations resulted in the finding that 24 were capable of hydrolyzing starch, a new finding. This unusual and perhaps significant characteristic, along with other taxonomic information led to the proposal of the new species, Lactobacillus amylophilus. These microorganisms represent new and unusual germplasm that may be of interest in studies concerned with starchy substrates. (See FL, D.1.)

Rapid Quantitative Method for Measuring Aflatoxin  $M_1$  in Dairy Products. In 1977, a serious problem of aflatoxin contamination occurred with corn in the southeastern states. In 1978, a similar problem was encountered with cottonseed in Arizona. In both instances, the damaged agricultural commodity was fed to dairy cattle and high levels of aflatoxin  $M_1$  were found in commercial milks. A rapid, economic quantitative method for measuring the  $M_1$  content in dairy products was developed because current methods were lengthy and costly. The method was chosen for testing in an international collaborative study conducted jointly by the Association of Official Analytical Chemists and the International Union of Pure and Applied Chemistry. It appears from the results



of the study that the method is acceptable. This will enable the fast monitoring of consumer dairy food products necessary to assure their safety. (See FL, G.1.)

The Fate of Mycotoxins in Contaminated Corn During Ethanol Fermentation. Corn naturally contaminated with the mycotoxin aflatoxin was utilized as a substrate in conventional alcohol fermentations. Distribution of aflatoxin in the various fractions demonstrated that the mycotoxin does not appear in the distilled alcohol but does accumulate in the spent grain. Little destruction of aflatoxin occurred during the alcoholic fermentation. The same procedure was used with zearalenone, an estrogenic mycotoxin, and the same results were obtained, namely that no zearalenone remained in the alcohol but was concentrated in the solids after fermentation without any appreciable reduction of total zearalenone. (See FL, G.1.)

Validation of Minicolumn Screening Methods for Aflatoxin in Corn. Three minicolumn rapid-screening methods for aflatoxin in corn were tested in a collaborative study with 19 laboratories participating. Laboratories were in state and federal agencies, universities, and industries. Samples included spiked and naturally contaminated corns from 2.5 to 85 ppb. The three methods were reliable for detecting 10 ppb aflatoxin in corn in all laboratories. Persons with more experience were capable of detecting 5 ppb. As a result of these studies, the method known as the Holaday-Valasco method was accepted in official first action by the Association of Official Analytical Chemists. It was also possible to eliminate two previous minicolumn methods from the AOAC Book of Methods. Minicolumn methods are widely used in the South and Southeast to detect aflatoxin in corn; e.g., 18,000 samples were run in 1 year in Georgia alone. Growers, elevator owners, and feed manufacturers depend on the results for marketing and feeding corn. Therefore, it was imperative that the reliability of the minicolumn methods used be established. (See FL, G.1.)

New Plant Hormone. In cooperative research with BARC and ERRC, a novel plant growth promoter, brassinolide, has been isolated, and the chemical structure has been established. Brassinolide stimulates elongation, swelling, and splitting of bean internodes. This combination of responses is unique and unexpected. Brassinolide represents the first steroid-type molecule to show growth-promoting activity in plants. Previously, steroids were well known as animal hormones, but no physiological activities were known in plants. Further research is required to learn what effect brassinolide or related compounds may have on increasing crop yields. [See Horticultural and Special Crops Laboratory (HSC), C.1.]

Glucosinolates in Cruciferous Vegetables Now Fingerprinted for Plant Breeders. For the first time, data are available to assist plant breeders in developing new cultivars of cabbage that will not inadvertently have increased levels of potentially toxic glucosinolates. All major cultivars of cabbage have been surveyed to establish current levels and patterns of the dozen glucosinolates that occur. Total glucosinolates range from 299 to 1,288 ppm in common cabbage (*Brassica oleracea*) and from 174 to 1,357 ppm in Chinese cabbage (*B. campestris*). This study is the result of constant close cooperation between plant breeders and chemists. (See HSC, F.2.)

Photosynthesis in Flashing Light. A method involving light flashes was discovered to detect the flow of electrons between the oxygen-producing and the carbon-fixing reaction centers in the green chloroplasts of plant material. The detected signals were generated during illumination with light flashes a few millionths-of-second long. Signals were shown to originate specifically from the carbon-fixing reaction center and were due to the generation of a form of oxygen called superoxide ( $O_2^-$ ). Also, this method can be used to alter the charge state of the electron carriers between the oxygen-producing and the carbon-fixing reaction centers. Out of such basic studies comes new concepts for increasing photosynthetic efficiency and for improving crop yields. [See Oilseed Crops Laboratory (OC), A.1.]

Method for Determination of Soy Protein in Meat-Soy Blends. A simple fluorometric method has been developed for determining the amount of soy protein in uncooked blends of beef and textured soy protein. Textured soy protein is used as an extender, and Government regulations limit the amount that can be added to various meat products. For example, in Europe, a major market for U.S. soybeans, some countries do not allow the addition of soy proteins to processed meats unless adequate analytical techniques are available to monitor the soy-extended products. This method therefore helps meet the need for an analytical technique to control the level of soy protein in meat products and to assure the consumer that soy extended products are correctly labeled. (See OC, B.1.)

Soybean Oil Flavor and Odor Deterioration. Taste panels and chemical researchers tell us that the flavors and odors of soybean oil are caused by reaction with oxygen of the air during storage and cooking. These changes vary with exposure to light and temperature. A basic study of the structure of oxygen products called hydroperoxides indicates they might account for the flavor deterioration of soybean oil. When individual pure fatty acids were reacted with oxygen, the hydroperoxide composition was the same at different levels of oxidation. In contrast, when the mixture of fatty acids which occurs in soybean oil was oxidized, an unusual distribution of hydroperoxide compounds as produced which varied significantly with different levels of oxidation. A sophisticated instrumental method--gas chromatography and computerized mass spectrometry--provides for the first time an answer to the basic question of which hydroperoxides contribute to the undesirable odors of soybean oil. This fundamental information on what causes its unique flavor and odor deterioration is required if soybean oil is to compete in export markets with peanut oil in France and olive oil in the Mediterranean countries. (See OC, B.2.)

Post-Harvest Handling of Soybeans: Mechanical Damage Affects Oil Quality. Split beans were demonstrated to have a disproportionate negative effect on oil quality. Crude oils extracted from soybeans at various points in the export marketing chain and from split bean fractions segregated therefrom, contain increased levels of deleterious constituents compared to oils from beans of the same shipment at the origin. Oil from split beans, relative to whole bean oil, was found to have increased levels of free fatty acids, which increase refining losses during processing, and iron, a catalyst for oxidation of the oil. During shipment, oil quality factors deteriorated with split beans but not with whole beans. (See OC, B.5.)



Tagged Dietary Fats Used in Human Studies. A new research technique has been developed which allows three different dietary fats to be simultaneously tracked through the bloodstream. The main advantages of this new approach are (1) that three times as much information can be obtained from each experiment, (2) the data are more accurate, and (3) the method is safe to use in human experiments. With this technique, the nontoxic stable isotope of hydrogen (deuterium) is used to tag three different fats found in partially hydrogenated soybean oil so that each fat contains a different level of deuterium. These tagged fats can be distinguished in blood samples by mass spectroscopy. The accuracy of the new methodology has been demonstrated, and the necessary experimental parameters and details have been established. Results indicate this new research tool has wide applicability to a variety of research problems that involve fat metabolism in humans. Nutrition, coronary heart disease, cystic fibrosis, and obesity are examples of medical and biochemical research areas where this new tool can be applied. (See OC, E.1.)



## CEREAL PRODUCTS LABORATORY

W. M. Doane, Acting Chief

Research Leaders: M. O. Bagby, B. T. Hofreiter, and F. H. Otey

### A. TECHNOLOGIES FOR INDUSTRIAL USES OF PLANT AND ANIMAL PRODUCTS

#### 1. Nonpollutant Paper and Textile Additives from Cereal Flours and Starches (B. T. Hofreiter and J. C. Rankin)

- a. Specific Objective: Optimize reaction conditions, reactant ratios, and types of starch to give the best acrylic acid copolymers for textile sizes. Use principles developed in sizing work, relating graft copolymer structures to properties to prepare starch derivative suitable as permanent textile sizes.

Progress: Reaction conditions including concentrations, reactant ratios, and starch type were assessed for their effects on add-on, homopolymer/grafted polymer ratios, and solution properties. Increasing reactant ratios of acrylic acid increased graft add-on from 6% (1:10, monomer:oxidized starch) to 20% (1:2). The solution viscosities were directly related to the amounts of grafted copolymer.

Abrasion testing performed on cotton and cotton polyester blends sized with solutions of these graft copolymers showed improved abrasion resistance with increases of acrylic acid add-on.

- b. Specific Objective: Continue studies on cereal-based cationic, anionic, and amphoteric graft copolymers prepared by dry-state reaction conditions that correlate intrinsic properties to performance as paper and textile sizes.

Progress: Seven acrylic-based monomers were reacted at various conditions of time, temperature, and pH with wheat starch and a corn flour using several free-radical initiator systems (chemical and ionizing radiation) to synthesize a large number of graft copolymers. The copolymers are being characterized in respect to structure-related parameters, add-on, and intrinsic viscosity, and correlation being made with performance properties in paper pulp slurries as interfiber bonding and retention agents. Results while not yet definitive in the structure-properties aspect, do indicate potential of the graft copolymers as dry-strength additives for paper.

- c. Specific Objective: Complete evaluation of quaternary cationic flours and starches (prepared with 3-chloro-2-hydroxypropyl-trimethylammonium chloride) in dry-end applications on the NRRC pilot paper machine. Continue preparative studies, characterizations, and evaluation of anionic starches and flours made with 3-chloro-2-hydroxypropyl sodium sulfonate.

Progress: The quaternary cationic flours and starches were concomitantly dispersed and enzymatically degraded for application as sizes for bond papers on the NRRC pilot paper machine. The pasted products imparted similar paper strength and surface properties benefits as those of commercial products made by conventional processes without any differences in runnability. However, the experimental sizes showed more effectiveness for pollution abatement (reduction of material losses in wastewater), improvement of paper properties, and potential for upgrading recycled papers. Strong interest continues both in the U.S. and abroad by potential suppliers and paper manufacturers in the dry processed cationic products because of energy conservation and pollution abatement benefits.

Reactions carried out under these same dry conditions employing chloro-2-hydroxypropyl sodium sulfonate showed very little reacted with starch.

- d. Specific Objective: Evaluation of new starch polyampholytes as retention and drainage aids in formation of paper and flocculants for the purification of effluents with high suspended-solids content.

Progress: Evaluation of a series of starch polyampholytes as strengthening additives for paper has been completed. In preliminary paper machine trials, these derivatives have been shown to dramatically improve drainage during formation of paper on the wire and to act as effective flocculants of suspended solids in the paper machine effluents. The cation/anion ratio and pH were found to be particularly important variables.

- e. Specific Objectives: Evaluate starch graft copolymers as sizing agents for textiles.

Progress: Several series of graft copolymers of starches with acrylic acid were prepared using chemical initiation with ferrous sulfate-peroxide and photoinitiation with a mercury vapor lamp. Studies of variables involved in these preparations led to the preparation of a series of graft copolymers from an oxidized starch and acrylic acid presumably suitable for textile sizing. Results of evaluations at the Southern Regional Research Center suggested that any such improvements of performance would be offset by additional cost and difficulties of drying such sizes on the looms because of the thickening tendency of grafted polyacrylic acid, even where low-viscosity oxidized starches would be used in the graft copolymers.

- f. Specific Objective: Preparation of dry-processed cationic cereals of reduced molecular weight for surface sizing by ionizing radiation.

Progress: A ready-to-use cereal derivative was prepared using dry processing throughout, thereby providing potential energy savings over conventional processes in either manufacture or in dispersion at the end-use site. Commercial cationic starches and a cationic corn flour were gamma-irradiated ( $^{60}\text{Co}$ ) to various degrees and evaluated



as bond paper surface sizes in pilot paper machine trials. Excellent results were obtained in both rheological and paper sizing properties.

- g. Specific Objective: Preparation and evaluation of reduced-viscosity carbamoylethylated starch (CES) as pigment coating and surface sizing adhesives with water resistance.

Progress: A series of CES derivatives of varying degrees of substitution has been prepared using an acid-modified starch and applied as pigment coating adhesives. CES-clay coatings reacted with hypochlorite to form water-insoluble paper coatings comparable to off-set and label-grade coated papers. Numerous variables associated with the preparation and application of these CES-based coatings have been identified and optimized. The use of CES derivatives for surface sizing is in progress and results are promising.

Publications:

CARR, M. E. AND B. T. HOFREITER. Ampholytic Starch Wet-End Additives. Staerke, in press.

CARR, M. E., B. T. HOFREITER, AND C. R. RUSSELL. Starch Polyampholytes with Amine and Xanthate Substituents: Characterization and Application. J. Appl. Polym. Sci. 22 (1978):897-904.

CHENG, W. C. AND M. E. CARR. O-Carboxymethylstarch Amine Polyampholytes as Papermaking Additives. Staerke 30 (1978):280-282.

HAMERSTRAND, G. E. AND M. E. CARR. Interpolymer Paper Strength Additives. U.S. Patent Allowed.

HAMERSTRAND, G. E., B. S. PHILLIPS, J. C. RANKIN, AND B. T. HOFREITER. Dry Processed Cationic Starches and Flours: Performance in Kraft Bag and Filled Bond Paper. Tappi 61 (1978):59-61.

HEATH, H. D. AND B. T. HOFREITER. Filler-Retention and Dry-Strength Additives. A Modified Handsheet Evaluation Procedure. Tappi 61 (1978):21-23.

HOFREITER, B. T., K. L. SMILEY, AND J. A. BOUNDY. Preparation of Retrogradation-Resistant Starches with Immobilized Amylases. U.S. Patent 4,121,974 (October 24, 1978).

HOFREITER, B. T., K. L. SMILEY, J. A. BOUNDY, C. L. SWANSON, AND R. J. FECHT. A Novel Modification of Cornstarch by Immobilized alpha-Amylase. Cereal Chem. 55 (1978):995-1006.

HOFREITER, B. T. AND R. G. FECHT. Improved Clarification-Sedimentation of Industrial Wastewaters Containing Starch Dispersants by Ionizing Radiation. Tappi, in press.

HOFREITER, B. T. Natural Products for Wet-End Additions. In Pulp and Paper--Chemistry and Technology, 3rd Edition, 3 Vol. Edited by James P. Casey, in press.

RANKIN, J. C. AND B. S. PHILLIPS. Low pH Preparation of Cationic Starches and Flours. U.S. Patent 4,127,563 (November 28, 1978).

TRIMNELL, D., E. I. STOUT, W. M. DOANE, AND C. R. RUSSELL. Preparation of Starch 2-Hydroxy-3-Mercaptopropyl Ethers and Their Use in Graft Polymerizations. J. Appl. Polym. Sci. 22 (1978):3579-3586.

#### Other Reports:

CARR, M. E., R. G. FECHT, AND B. T. HOFREITER. Chlorinated Carbamoyl-ethyl Starch for Wet-Rub Resistant Paper Coatings. Presented at TAPPI Coating Conference, Cincinnati, Ohio, May 21-23, 1979.

RANKIN, J. C. Dry-State Preparation and Properties of Quaternary Cationic Flours and Starches. Presented at the Annual Corn Dry Milling Conference, NRRC, Peoria, Illinois, June 1, 1978.

### 2. Starch-Based Copolymers for Making Elastomers (T. P. Abbott)

- a. Specific Objective: Determine if the reaction of a surfactant-free, sulfonic acid containing elastomer with a basic amine modified starch can yield an ionically bonded composite with better physical properties than existing composites of carboxy nitrile elastomer and basic amine modified starch.

Progress: Several new combinations of a styrene-butadiene-(styrene sulfonic acid) terpolymer (SB-SO<sub>3</sub>) in salt form with an amine-modified starch in the chloride form gave composites with minimal tensile strengths. Combinations of carboxy nitrile elastomers with basic amine modified starches still gave the best properties of those studied so far.

Problems encountered in making the SB-SO<sub>3</sub> in a surfactant free latex form led to the discovery that styrene sulfonic acid forms a water soluble copolymer with styrene that can act alone as an emulsifier in the further terpolymerization of styrene-butadiene rubber.

#### Publication:

ABBOTT, T. P., C. JAMES, AND F. H. OTEY. Ionically Bonded Filler-elastomer Composites. J. Appl. Polym. Sci. 23 (1979):1223-1231.

### 3. Starch-Based Purification Aids For Wastewater (W. M. Doane)

- a. Specific Objective: Determine the influence of molecular weight and type of charge of starch polymer on flocculating ability.

Progress: Several water-soluble starch-based flocculants were prepared. The effect of charge (anionic or cationic) and molecular



weight (low to high) showed little influence on flocculating ability. Because of the highly branched structure of starch, a large excess of the starch-based flocculating agent was required to get comparable flocculation as with high molecular weight straight chain synthetic polymers. Several water-insoluble starch-based products containing metal chelating groups were prepared and tested as heavy metal scavengers. Some of these products show excellent potential for removing heavy metal ions from solution.

- b. Specific Objective: Prepare and evaluate water-soluble starch-based polymers as flocculating and sludge dewatering agents to provide water suitable for irrigation.

Progress: Several water-soluble starch-based flocculants with different charges were prepared and evaluated for their ability to flocculate suspended solids from suspensions. The effect of molecular weight of the initial starch used had no noticeable effect on flocculating ability. Insoluble starch xanthate (ISX) has been commercialized and discovered to be a good polymer for removing heavy metals from water, and our U.S. Patent 3,979,286 on ISX has been licensed by 16 companies. In September 1978 NRRC received awards from Pollution Engineering Magazine and Industrial Research/Development Magazine (IR-100) for the discovery of ISX. Many companies are preparing insoluble starch xanthate and are using it to scavenge the last traces of heavy metals before discharging their effluent water.

Publications:

NAVICKIS, L. S., R. E. WING, AND E. B. BAGLEY. ISX Reduces Selenium Count in Process Wastewaters. Ind. Wastes 25 (1979):26-30.

RAYFORD, W. E., R. E. WING, AND W. M. DOANE. Carboxyl-Containing Starch Graft Polymer: Preparation and Use in Heavy Metal Removal. J. Appl. Polym. Sci., in press.

WING, R. E., W. E. RAYFORD, AND W. M. DOANE. Treatment of Electroless Nickel Plating Rinse Waters. Met. Finish. 76 (1978):54-57.

WING, R. E. Processes for Heavy Metal Removal from Plating Wastewaters. First Annual Conference on Advanced Pollution Control for the Metal Finishing Industry. EPA-600/8-78-010, (1978):96-106.

WING, R. E. AND W. E. RAYFORD. Use of Spent Pickle Liquor in Waste Treatment. Evaluation for Electroless Copper and Copper Etchant. Met. Finish. 76 (1978):31-33.

WING, ROBERT E. AND WILLIAM M. DOANE. Removal of Heavy Metal Ions from Aqueous Solutions with Insoluble Crosslinked-Starch Xanthates. U.S. Patent 4,083,783 (April 11, 1978).

WING, R. E. Chemical Waste Treatment Processes for Minimum Discharge of Pollutants from the Printed Circuit Industry. Proceedings of IPC Workshop on Water Pollution Control, Chicago, IL. 1978.

WING, R. E. Treatment Processes for Electroplating Rinse Waters. Proceedings of the American Institute of Chemical Engineers, Miami Beach, FL. 1978.

WING, R. E. Treating Plating Waste with ISX. Insoluble Starch Xanthate Shows Promise in Ion Exchange Removal of Heavy Metals. Ind. Finish. 54 (1978):38-40.

WING, R. E., W. E. RAYFORD, AND W. M. DOANE. Treatment of Complexed Copper Rinsewaters with Insoluble Starch Xanthate. Plat. Surf. Finish. 65 (1978):52-57.

WING, R. E., W. E. RAYFORD, W. M. DOANE, AND C. R. RUSSELL. Preparation of Insoluble Cationic Starches and Their Use in Heavy Metal Anion Removal. J. Appl. Polym. Sci. 22 (1978):1405-1416.

#### Other Reports:

WING, ROBERT E., LEO L. NAVICKIS, BRIAN K. JASBERG, AND WARREN E. RAYFORD. Removal of Heavy Metals from Industrial Wastewaters Using Insoluble Starch Xanthate. EPA Interagency Agreement Final Report. (Technical Research Report.) EPA-600/2-78-085 (1978).

#### 4. Flame-Resistant Polyurethane Foams and Biodegradable Films and Packaging Prepared from Starch (F. H. Otey)

- a. Specific Objective: Continue a systematic approach to find an optimum method for plasticizing starch films with the ultimate aim of establishing a correlation between the chemical structure of polyols and their effectiveness as plasticizers for starch films.

Progress: 1,2-Dihydroxy ethane, 1,3-dihydroxy propane, 1,2,3-trihydroxy propane, 1,2,4-trihydroxy butane, and 1,2,6-trihydroxy hexane were used alone as plasticizing agents for alkali-gelatinized starch, or in combination with butanediol diglycidyl ether. Films cast from the combinations varied in trend of physical strength characteristics as compared to those cast with polyol alone, thus for example, in the cases of 1,2-dihydroxy ethane and 1,2,3-trihydroxy propane the presence of the epoxide raised the percent elongation but decreased the break load and the tensile strength whereas in the case of 1,2,4-trihydroxy butane the presence of the epoxide raised all three strength characters.

Comparing film strength data within the groups of diols and triols, with the epoxide added, it was noted that as the carbon chain distance between hydroxyl groups increased in the diols, the percent elongation dropped but the break load and the tensile strength increased; and the same strength change progressions were noted in the triol group as the distance between the 1,2 dihydroxy grouping and the omega hydroxyl group increased.

Films cast from alkali-gelatinized starch with glycerol and long-chain, aliphatic, resinous epoxide mixtures as plasticizers were



found superior in percent elongation and tensile strength characteristics compared to those cast with glycerol and relatively short chain aliphatic or aromatic diepoxide monomers.

- b. Specific Objective: Evaluate starch-polyvinyl alcohol films that have been plasticized with various polyols and correlate the effects of aging on the flexibility and strength of the films with the structure of the various polyol plasticizers.

Progress: Mixtures composed of 51% starch, 17% polyvinyl alcohol (PVA), 1% paraformaldehyde, 1%  $\text{NH}_4\text{Cl}$ , and 30% of various plasticizers were dispersed with heat into water and cast into clear films. Eighteen different plasticizer formulations were evaluated. Films were tested for percent elongation (E) and tensile strength (T) after aging, generally 1 day, 43 days, 240 days, and in some instances up to 620 days. Various equations were fitted to the data with E or T as the dependent variable and  $t$  (age of films in days) as the independent variable. The data revealed that films containing 30% glycerol (the most commonly used plasticizer for starch films) are very flexible and relatively weak when first prepared, but the effect of the plasticizer decreases rapidly with aging. Preliminary tests with solvent extraction suggested that the glycerol becomes associated with starch or PVA hydroxyls and ceases to be an effective humectant. Much more stable plasticizing was achieved using 7.5% glycerol in combination with 22.5% glycol glycoside plasticizer, where both T and E were essentially unaffected throughout a 220-day aging period. Also, combinations of glycerol and sorbitol or glycerol glycosides were effective plasticizers for starch films even after a year or more of aging. Sucrose and glucose were poor plasticizers but offered some promise when used in combination with sorbitol or glycerol.

Improved plasticizer systems for starch films are especially important now that starch has become increasingly attractive as a raw material in film production because of decreasing availability of petroleum-based resins and the need for biodegradable films.

- c. Specific Objective: Continue evaluation of starch-ethylene acrylic acid copolymer films for mulching and packaging applications.

Progress: Various types and amounts of starch were combined with ethylene acrylic acid copolymer in a Brabender Plastograph and then extrusion processed through a 1-inch wide die to yield a clear plastic ribbon. The various types of starch evaluated included ordinary corn starch, irradiated starch, and pregelatinized and benzoyl ethers of starch. Ordinary corn starch proved to be the most effective based on economics and plastic quality. As the level of starch was increased from 30% to 90%, the tensile strength of the plastic ribbon increased from  $166 \text{ kg/cm}^2$  to  $295 \text{ kg/cm}^2$ , and the percentage elongation decreased from 160% to 5.1%. These plastics have potential application in the production of biodegradable planters for automatic transplanting operations of field crops.

By studying the interaction between starch and synthetic polymers, ways were discovered for extruding combinations of starch and ethylene acrylic acid copolymers that yielded films having properties that closely resemble those of polyethylene. The films are biodegradable after extended periods of soil exposure, depending upon the composition, and they do not require a water-soluble plasticizer. The study is believed to have overcome major obstacles leading to a feasible biodegradable plastic needed for agricultural applications. Small test samples of the films are being evaluated by USDA scientists in Texas and Florida in connection with programs on automatic transplanting systems for field crops and the field seeding of crops conventionally transplanted.

#### Publications:

OTEY, F. H., C. A. WILHAM, AND C. R. RUSSELL. Flame-resistant Polyurethane Foams Derived from Methyl Glucoside Polyethers. Ind. Eng. Chem., Prod. Res. Dev. 17 (1978):162-164.

WESTHOFF, R. P., W. F. KWOLEK, AND F. H. OTEY. Starch-polyvinyl Alcohol Films--Effect of Various Plasticizers. Staerke, in press.

#### Other Reports:

ANONYMOUS. Plastics of the Near Future Will Offer Biodegradability. Plast. Des. Process. 18 (1978):50-54.

ANONYMOUS. Plastic May Save Oil, Trash. Montgomery Advertiser J., April 17, 1978.

ANONYMOUS. New Plastic Dissolves into Soil and Saves Oil. The Arizona Republic, April 17, 1978. D-6.

ANONYMOUS. Soluble Plastic on Drawing Board. The Times-Picayune, April 17, 1978.

ANONYMOUS. Dissolvable Plastic Eyed by Scientists. The Washington Post, April 17, 1978.

ANONYMOUS. Two Work on Dissolvable Plastics. Chicago Sun-Times, April 18, 1978.

ANONYMOUS. Biodegradable Wrappers Near. Environment News, June 1978.

FIELDS, DAVID. Center Finds More Uses for Crops. Ames Daily Tribune, Weekender Edition, October 21, 1978.

KIEFER-BELL, JULIE. Cornucopia--New Ingredient for Plastic. Summer 1978, p. 20.

VALMASSEI, CHRISTINE. They Make Unusual Things for You from Farm Products. Family, May 1978.



## 5. Survey of Domestic Plants for Rubber and Hydrocarbons (R. A. Buchanan)

- a. Specific Objective: Identify U.S. plant species that give promise as potential multi-use hydrocarbon producing crops.

Progress: In the continued screening of plant species collected throughout the United States, 100 additional species were evaluated this period. Out of the total of 300 species evaluated to date, 34 species representing 27 genera and 13 families were rated as offering some promise and grouped as potential rubber crops (13 species), rubber plus oil crops (11 species), whole-plant oil crops (8 species), and gutta crops (2 species). The most promising of the newly evaluated species were Lonicera tartarica (red tartarian, honeysuckle) for rubber and Euphorbia pulcherima for oil. Rated somewhat lower from the multi-use standpoint, but of interest as potential gutta producing crops were two Gramineae (grasses), Agropyron repens (quack grass), and Elymus canadensis (wild rye).

- b. Specific Objective: Characterize oils, polyphenols, and hydrocarbon polymers produced by select U.S. plant species.

Progress: Molecular weight distributions of natural rubbers (NR) from several additional species were measured. None of the newly examined species produced NR with high molecular weights comparable to Hevea or Guayule rubbers.

Gutta polymers from a group of Gramineae (grasses) having common biochemical characteristics were definitively characterized. Weight-average molecular weights ( $\bar{M}_w$ ) of these polymers ranged from 111,000 to 176,000. While these guttas have  $\bar{M}_w$  lower than those of most NR, they are high enough for the more crystalline trans-polymer to have useful properties. Grass guttas could have large-scale applications as thermoplastics and thermosetting resins.

Lipid compositions were estimated for whole-plant oils from 14 productive species. Most of these oils had a large component of nonglyceride esters that would be valuable feedstocks for producing such chemical intermediates as esters and waxes, long-chain alcohols, sterols, rosin and fatty acids, and terpenes.

- c. Specific Objective: Evaluate oils and hydrocarbons from select U.S. plant species for potential utility in rubber compounding.

Progress: A large sample of Asclepias syriaca (common milkweed) was collected and oil and hydrocarbon fractions were extracted and partitioned to give products compatible with natural rubber (NR) and styrene-butadiene synthetic rubber (SBR). Initial compounding studies yielded promising results.

- d. Specific Objective: Develop improved methods for extraction of oils, polyphenols, hydrocarbon polymers, and other products from U.S. plant species.

Progress: A novel process was developed and patented for the extraction of botanochemicals from plant materials. A scenario was developed for an integrated U.S. botanochemical production system based on relatively small scale processing of new multi-use crops. Potential processes, production economics, and marketing relationships were examined in a preliminary fashion.

Publications:

BUCHANAN, R. A. Extraction of Rubber or Rubberlike Substances from Fibrous Plant Materials. U.S. Patent 4,136,131 (January 23, 1979).

BUCHANAN, R. A., I. M. CULL, F. H. OTEY, AND C. R. RUSSELL. Hydrocarbon- and Rubber-Producing Crops: Evaluation of U.S. Plant Species. *Econ. Bot.* 32 (1978):131-145.

BUCHANAN, R. A., I. M. CULL, F. H. OTEY, AND C. R. RUSSELL. Hydrocarbon- and Rubber-Producing Crops: Evaluation of 100 U.S. Plant Species. *Econ. Bot.* 32 (1978):146-153.

BUCHANAN, R. A. AND F. H. OTEY. Multi-Use Oil- and Hydrocarbon-Producing Crops in Adaptive Systems for Food, Material and Energy Production. Proceedings of the International Conference "Larrea: Un Vasto Recurso de Los Desiertos Americanos," in press.

BUCHANAN, R. A., F. H. OTEY, C. R. RUSSELL, AND I. M. CULL. Whole-Plant Oils, Potential New Industrial Raw Materials. *J. Am. Oil Chem. Soc.* 55 (1978): 657-662.

SWANSON, C. L., R. A. BUCHANAN, AND F. H. OTEY. Molecular Weights of Natural Rubbers from Selected Temperate Zone Plants. *J. Appl. Polym. Sci.* 23 (1979):743-748.

BUCHANAN, R. A., C. L. SWANSON, D. WEISLEDER, AND I. M. CULL. Gutta-Producing Grasses. *Phytochemistry*, in press.

Other Reports:

BUCHANAN, R. A. AND F. H. OTEY. Potential Multi-Use Crops for Adaptive Systems of Food, Material, and Energy Production. Presented at Nineteenth Annual Meeting, The Society for Economic Botany, St. Louis, Missouri, June 11-14, 1978.

BUCHANAN, R. A. Oil and Hydrocarbons from Plants. Presented in the "Wednesday Night at the Lab." lecture series. University of Illinois, September 20, 1978.

International news coverage via radio, television, newspapers, and trade and farm magazines resulting in telecasts, broadcasts, stories, articles, and features too numerous to list.



6. Controlled-Release of Pesticides by Formulating with Cereal-Based Starch and Flour Derivatives (W. M. Doane)

- a. Specific Objective: Study variables in preparation of starch encapsulated pesticides to optimize performance of formulated products.

Progress: Continued research on encapsulating pesticides in a starch matrix to provide controlled release formulations has resulted in some significant improvements. Crosslinking the starch xanthate with a sulfuric acid-hydrogen peroxide system allows preparation of encapsulated products with a much higher loading of active agent. Products with as much as 50% by weight of chemical pesticide have been prepared and are nonsticky. Retention of the pesticide during encapsulation is also improved. The modified crosslinking system also allows use of a starch xanthate with fewer xanthate groups thereby improving the economics of this process. Shelf life of encapsulated insecticides, which are susceptible to hydrolysis, has been improved by incorporating a small amount of a desiccant into the granular encapsulated material.

- b. Specific Objective: Prepare a series of modified starch compounds containing covalently bonded pesticides attached through ester, acetal, imide, and other linkages, and determine efficacy of formula-tive products for controlling pests.

Progress: A number of starch esters of picloram of varying degree of substitution were prepared. One of these products prepared from a pregelatinized starch containing 39.4% picloram was used in hydrolysis studies on the release of the active ingredient. This was determined by a biological method using lettuce as the target plant. Hydrolysis systems containing an unsterilized soil aided in the release of the picloram so that aliquots removed after 12 days or more and applied to sterilized soil seriously retarded lettuce growth for at least three reseeds. New seedlings were made 16 days after the previous one.

A parallel study using the alcohol insoluble material of the starch ester of picloram gave similar results verifying the actual release of the active ingredients. Alcohol extraction removes any free picloram that may be present in the product.

Reactions between tolylene 2,4-diisocyanate and herbicides such as metrilrizin, picloram, and amiben were successful but these products did not react with starch under the conditions studied.

- c. Specific Objective: Continue to cooperate with SEA-AR, industrial, and university scientists on evaluation of the starch-based slow release herbicides, insecticides, and nematicides.

Progress: Cooperation with many SEA-AR weed scientists, entomologists, and nematologists, and with scientists at universities and industry continued at a high level. NRRC chemists prepared numerous starch

encapsulated pesticides for greenhouse and field testing by the cooperating scientists. Feedback received during the tests was used to modify release properties of the encapsulated products to improve their efficacy in pest control. Several of the cooperators are presenting and publishing their findings. Cooperator R. Miller, SEA-AR, at Beltsville, got good results with the larvicide, Dimilin, when it was encapsulated. Cellulose xanthate was better than starch xanthate as the encapsulating matrix, since eggs produced by chickens fed the cellulose encapsulated Dimilin contained smaller amounts of the larvicide than did those when starch was used. Cooperator J. Dale, SEA-AR, Stoneville, Mississippi, found enhanced efficacy of the herbicide, Sencor, when encapsulated in the starch matrix. Several SEA-AR cooperators (Coffman and Gentner at Beltsville, Wax at Urbana, and Schreiber at Purdue) all recorded excellent results with the herbicide Treflan encapsulated in crosslinked starch xanthate. Losses due to volatility and light decomposition are greatly reduced for the encapsulated products.

Starch encapsulated picloram and Esteron give better weed control than standard commercial formulations of these two herbicides as shown by H. Moyeux at Temple, Texas, and J. Baur at College Station, Texas.

FY 1978 was the third consecutive year of cooperation with M. Schreiber at Purdue and J. Feldmesser at Beltsville. Close cooperation with these researchers has resulted in advancing the controlled release formulation of thiocarbamate herbicides (Schreiber) and nematicides (Feldmesser).

A paper written by AR entomologists E. Herbert and H. Shimanuki at Beltsville describes improved brood rearing and feed consumption by bees fed an artificial diet containing pollen attractants encapsulated in a starch matrix.

- d. Specific Objective: Continue to evaluate various polymeric additives to sprayable formulations for foliar application to provide insoluble films which will entrap the pesticide and increase its effectiveness.

Progress: Samples of methanol treated activated sludge polymer (MAS) containing pesticide and dialdehyde starch were sent to 10 cooperating USDA, Extension and industrial research scientists for field testing and evaluation. Results were somewhat variable and inconclusive. Contrary to earlier preliminary results, field tests this year did not show improved efficacy for most herbicides applied along with MAS polymer. Some tests at the University of Illinois did show slight improvement in crabgrass control in the presence of MAS. Scientists at Rutgers reported that MAS polymer prevented foliar injury to Kentucky bluegrass treated with high rates of maleic hydrazide and embarks (growth retardants).



## Publications:

ROTH, WILLIAM B. Methanol Treated Activated Sludge as an Agricultural Chemical Carrier. U.S. Patent 4,065,287 (December 27, 1977).

SCHREIBER, M. M., B. S. SHASHA, M. A. ROSS, P. L. IRWICK, AND D. W. EDGECOMB. Efficacy and Rate of Release of EPTC and Butylate from Starch Encapsulated Formulations Under Greenhouse Conditions. *Weed Sci.* 26 (1978):679-686.

SHASHA, B. S. Methods for Testing and Factors Affecting Rate of Release of Encapsulated Products Prepared via Starch Xanthate. Proceedings of 1978 Controlled Release Pesticide Symposium.

STOUT, E. I., B. S. SHASHA, AND W. M. DOANE. Pilot-Plant Process for Starch Xanthide Encapsulated Pesticides. *J. Polym. Sci.*, in press.

## Other Reports:

MAYEUX, H. S., C. W. RICHARDSON, R. W. BOVEY, R. E. MEYER, E. BURNETT, AND B. S. SHASHA. Slow Release Formulation Alters Picloram Residue Patterns. Presented at 32nd Annual Southern Weed Science Society at Atlanta, Georgia, January 23, 1979.

## 7. Graft Polymers of Starch for Agricultural Chemicals and Absorbents (G. F. Fanta)

- a. Specific Objective: To better understand the mechanism of water swelling of saponified starch graft copolymers and other gels.

Progress: A series of crosslinked polyacrylamide gels were prepared for use as models to better understand the rheology and swelling behavior of saponified starch-polyacrylonitrile gels (Super Slurper). Gels were prepared with various amounts of N,N'-methylenebisacrylamide as crosslinking agent, under conditions such that a very high molecular weight product would result if no crosslinking agent were present. This procedure minimizes the number of free chain ends in the gel product. The gels were then cut into small pieces to make a paste. The most effective method, better than dry grinding, was to feed the wet gel through a chopper with a small-hole cutter plate. The gel particles were screened wet through 60 mesh, and washed with water to remove soluble polymer and contaminants. Rheological properties--elastic and loss moduli, and viscosity--as well as water swelling properties of the refined gel products were determined.

A small fraction of the carboxamide groups in the gels were then hydrolyzed to form carboxyl groups, and the resultant polyelectrolyte gels were reexamined and compared to the original gels. Satisfactory procedures have been developed for this study and a few gels have been examined. Values of elastic modulus are especially instructive and appear to be divisible into separate components representing a number of cross linkages and polymer entanglements.

- b. Specific Objective: Compare the evaporation of water from Super Slurper dispersions with the evaporation of water from soil and clay. Determine the lowest water content of Super Slurper that will release water to a seed in a growth medium.

Progress: Available moisture in sand-Super Slurper mixtures was determined with a moisture meter in 10-inch clay pots equipped with soil blocks at mid-level. Meter readings showed that addition of low levels of Super Slurper (0.04% and 0.14%, by weight) would double and triple the number of days during which the available moisture in the sand remained at 100%. Less dramatic results were obtained when top soil was substituted for sand. Weight loss measurements were used to compare the evaporation of water from 0.05% Super Slurper in water and from 0.5% peat-top soil (50:50) in water with the evaporation rate of water alone. The Super Slurper mixture had the same evaporation rate as water, while the evaporation of water from the peat-top soil mixture was somewhat faster, presumably due to its higher surface area. Evaluations of agar-Super Slurper mixtures in yeast and other media indicated that such mixtures would not be suitable for streak plates due to insufficient rigidity of the gel. However, these gels might well serve as satisfactory supports for growing microorganisms or for use in tissue culture.

- c. Specific Objective: Examine the extrusion of starch poly(methyl acrylate) graft copolymers and study the physical properties of the resulting plastics.

Progress: Starch-g-poly(methyl acrylate) [S-g-PMA] copolymers have negligible resistance to solvents for PMA. Extruded ribbons disintegrate within half an hour when immersed in benzene, acetone, glacial acetic acid, or ethyl acetate. Water immersion causes whitening and weakening but not disintegration. Nonsolvents such as kerosene or butanol have little effect on appearance or strength properties of the graft copolymer. Tensile strength of graft copolymers after removal of homopolymer is little affected by percent add-on of PMA over the range 43-64%. However, partial removal of starch by enzyme treatment reduced tensile strengths of the copolymers by as much as two-thirds. S-g-PMA copolymers contain varying amounts of cross-linked PMA which is insoluble in common PMA solvents. Removal of starch from graft copolymers by periodate oxidation, perchlorate oxidation, enzyme treatment, or acid hydrolysis always leaves a benzene-insoluble residue. This insoluble portion hinders measurement of molecular weights of grafted PMA for correlation with observed physical properties. Elasticity of S-g-PMA was improved by partial acetylation of the starch component.

- d. Specific Objective: Prepare carbohydrate-containing graft copolymers which will function as absorbents for organic liquids.

Progress: Mixtures of acrylamide and 2-acrylamido-2-methylpropanesulfonic acid (AASO<sub>3</sub>H) were graft polymerized onto starch by irradiating starch-monomer mixtures in water with cobalt-60. The resulting graft



copolymers were characterized as to synthetic polymer content (percent add-on), the percentage of  $\text{AASO}_3\text{H}$  in the synthetic moiety, and the molecular weight of the synthetic moiety. A second series of starch graft copolymers was prepared via ceric ammonium nitrate initiation from mixtures of  $\text{AASO}_3\text{H}$  and acrylonitrile, and the graft copolymers were similarly characterized. Reaction of these sulfonic acid containing starch graft copolymers with an ethoxylated fatty amine yielded polymeric amine salts which would absorb up to about 65 times their weight of 95% ethanol, depending on the  $\text{AASO}_3\text{H}$  content of the graft copolymer.

#### Publications:

DOANE, W. M. Polymer. McGraw-Hill Yearbook of Science and Technology (1978):301-303.

DOANE, W. M. Starch: Renewable Raw Material for the Chemical Industry. J. Coatings Technol. 50 (1978):88-98.

FANTA, G. F., R. C. BURR, W. M. DOANE, AND C. R. RUSSELL. Graft Polymerization of Vinyl Acetate onto Starch. Saponification to Starch-g-Poly(Vinyl Alcohol). J. Appl. Polym. Sci. 23 (1979):229-240.

FANTA, G. F., R. C. BURR, W. M. DOANE, AND C. R. RUSSELL. Absorbent Polymers from Starch and Flour Through Graft Polymerization of Acrylonitrile and Comonomer Mixtures. Staerke 30 (1978):237-242.

FANTA, G. F., F. L. BAKER, R. C. BURR, W. M. DOANE, AND C. R. RUSSELL. Scanning Electron Microscopy of Saponified Starch-g-Polyacrylonitrile. Staerke 29 (1977):386-391.

GUGLIEMELLI, L. A., C. L. SWANSON, W. M. DOANE, AND C. R. RUSSELL. Preparation of Starch Poly(Styrene-Co-Acrylonitrile) Graft Polymer by Cerium(IV) Initiation. J. Polym. Sci., Polym. Lett. Ed. 15 (1977):739-743.

GUGLIEMELLI, L. A., W. M. DOANE, AND C. R. RUSSELL. Preparation of Soapless Latexes by Sonification of Starch-Based Poly(Isoprene-Co-Acrylonitrile) Graft Reaction Mixtures. J. Appl. Polym. Sci. 23 (1979):635-644.

TAYLOR, N. W., G. F. FANTA, W. M. DOANE, AND C. R. RUSSELL. Swelling and Rheology of Saponified Starch-g-Polyacrylonitrile Copolymers. Effect of Starch Granule Pretreatment and Grafted Chain Length. J. Appl. Polym. Sci. 22 (1978):1343.

TAYLOR, N. W. AND E. B. BAGLEY. Stress Yield Value in Closely Packed Gel Suspensions. J. Appl. Polym. Sci., in press.

WEAVER, M. O., R. R. MONTGOMERY, L. D. MILLER, V. E. SOHNS, G. F. FANTA, AND W. M. DOANE. A Practical Process for the Preparation of Super Slurper, a Starch-based Polymer with a Large Capacity to Absorb Water. Staerke 29 (1977):413-422.

FANTA, G. F., E. I. STOUT, AND W. M. DOANE. Highly Absorbent Graft Copolymers of Polyhydroxy Polymers, Acrylonitrile, and Acrylic Comonomers. U.S. Patent 4,134,863 (January 16, 1979).

FANTA, G. F. AND W. M. DOANE. Increasing Absorbency of Polymeric Compositions by Curing. U.S. Patent 4,116,899 (September 26, 1978).

## B. TECHNOLOGIES FOR FIBER USES

### 1. Storage and Processing of Kenaf for Pulp and Papermaking (M. O. Bagby)

- a. Specific Objective: Prepare mechanical, thermomechanical, and chemi-mechanical kenaf pulps.

Progress: An industrial cooperator was identified and hired to defiber kenaf under several high-yield pulping conditions, to seek preferred conditions, and to produce sufficient quantities at the best conditions to allow pilot papermachine tests.

- b. Specific Objective: Produce chemical kenaf pulp for use in formulation of furnishes containing large proportions of kenaf fiber.

Progress: An industrial cooperator (by hire) prepared kenaf soda pulp and subsequently generated 3/4 ton of 78% brightness pulp. Bleaching was with a three-stage sequence, chlorine-chlorine dioxide, alkali extraction, and sodium hypochlorite. One hundred kg of kenaf pulp was retained unbleached.

- c. Specific Objective: Coordinate activities and advise cooperators on program leading to formation of 100 tons of newsprint and 50,000 tons in subsequent year.

Progress: American Newspaper Publishers Association (ANPA), a consumer association cooperator, with SEA-AR technical assistance has grown and harvested 30 acres of kenaf. Potential industrial cooperators were identified for the consumer group and SEA-AR has provided 125 lb of kenaf for preliminary trials.

- d. Specific Objective: Initiate the evaluation of other sources for fiber potential.

Progress: Guayule (Parthenum argentatum) is, again, receiving considerable attention as a domestic source of rubber. Consequently the fibrous stem was examined for fiber characteristics and gross chemical composition. Processing by the soda process yielded about 30% pulp. The liberated fibers were appreciably shorter than those of most hardwoods.



- e. Specific Objective: Continue experiments with kenaf on strip-mine land to broaden data base.

Progress: A cooperator was identified and a memorandum is being negotiated.

- f. Specific Objective: Coordinate continuing studies on kenaf with cooperating plant geneticists, agronomists, and agricultural engineers.

Progress: Kenaf was experimentally harvested with an Australian sugarcane harvester. While kenaf did wrap on rotating parts, harvesting proceeded unencumbered to give 30 cm billets.

Four varieties of kenaf were grown under five conditions of salinity by cooperating SEA-AR soil scientists and agronomists. The influence of salinity on fiber and chemical characteristics is being determined.

Chopped kenaf was stored under cover in 12-15 ton stacks for 1 year at about 70 and 30% initial solids. The former revealed little loss of solids, while, losses were appreciable for the latter. Treatments included borax and sodium bi-sulfite as potential preservatives. In contrast with those not chemically treated, both afforded improved preservation of solids stored at high moisture.

#### Publications

CUNNINGHAM, R. L., T. F. CLARK, AND M. O. BAGBY. *Crotalaria juncea*--Annual Source of Papermaking Fibers. *Tappi* 61 (1978):37-39.

DaVIA, D. J., M. O. BAGBY, B. A. WOLF, P. F. KNOWLES, AND J. R. GOSS. Cubing Increases Density of Kenaf. *Calif. Agric.* 32 (1978):11-12.

BAGBY, M. O., T. F. CLARK, W. C. ADAMSON, G. A. WHITE, AND R. L. CUNNINGHAM. Bale Storage of Kenaf for Pulp. TAPPI Press Report, Non-Wood Plant Fiber Pulping, Progress Report No. 9 (1978):33-39.

CUNNINGHAM, R. L., G. F. TOUZINSKY, AND M. O. BAGBY. Brightening of Kenaf Thermomechanical Pulp. *Tappi*, in press.

TOUZINSKY, G. F. AND S. H. GORDON. Degree of Substitution of Cellulose Derivatives Containing Different Substituent Groups. *Carbohydr. Res.*, in press.

#### Other Reports:

CUNNINGHAM, R. L. AND M. O. BAGBY. Sodium Hypochlorite Bleaching of Kenaf Soda Pulp. Abstract of Papers 71st Annual Meeting Illinois State Academy of Science, Normal, Illinois, April 21-22, 1978, 7.

BAGBY, M. O. Kenaf for Newsprint--Need, Production, Composition, Harvesting, Handling, and Papermaking. Invited presentation, American Newspaper Publishers Association, Reston, Virginia, NRRC, Peoria, Illinois, January 25, 1978.

BAGBY, M. O. A New Look at Agriculture for Coating Raw Material. Invited presentation, Cleveland Society for Coating Technology, Independence, Ohio, April 11, 1978.

BAGBY, M. O. Kenaf Newsprint--A Critique, Invited presentation. American Newspaper Publishers Association, Canadian International Paper, and International Paper, Hawkesbury, Ontario, Canada, June 15, 1978.

BAGBY, M. O. New Crops-Application to Stripmined Lands. Invited presentation, Big Bluestem Advisory Committee, Canton, Illinois, February 9, 1979.

Numerous interviews with newspapers, technical journals, trade journals, television, and radio reporters.



CEREAL SCIENCE AND FOODS LABORATORY

G. E. Inglett, Chief

Research Leaders: C. W. Blessin, J. E. Hodge, and J. S. Wall

A. TECHNOLOGIES FOR FOOD AND FEED  
USES OF FIELD CROPS

1. Enzyme Modification of Feed and Cellulosic Materials for Improved Beef Animal Feed (C. W. Blessin)

- a. Specific Objective: Improve Sephadex adsorption techniques for isolating the  $C_1$  and cellobiase cofactors of T. viride cellulase complex.

Progress: A procedure involving adsorption chromatography of T. viride cellulase preparations on Sephacryl S-200 columns has been successfully employed to separate and pool the cofactor necessary for the enzyme's efficient conversion of cellulose to glucose. Preliminary analytical data indicate that the organic fraction of the cofactor is an unsaturated carbon-hydrogen chain with a molecular weight of less than 1000. Precise chemical identification of the cofactor has been complicated by its instability (polymerization which forms insoluble products) and by its contamination with other low molecular weight compounds.

- b. Specific Objective: Continue examination of selected fractions of agricultural residues for their neutral carbohydrate composition and compare the composition of cobs and husks from corn varieties grown at different locations.

Progress: Pith from stalks of corn, sugarcane, sweet sorghum, and sunflowers was fractionated into hemicellulose components, and the neutral sugar content of each material was determined by gas-liquid chromatography. To compare pith with other components of the stalk, the rind and fibrovascular bundles of corn were fractionated and analyzed in the same manner. The neutral carbohydrate composition of corn husks and cobs from 13 single cross hybrids grown at 10 locations is being investigated. Only minor differences in the neutral carbohydrate composition were detected in the hybrids grown at different locations. Data on the effect of variety are not yet complete.

Publication:

JONES, R. W., L. H. KRULL, C. W. BLESSIN, AND G. E. INGLETT. The Neutral Sugars of Hemicellulose Fractions of Pith from Stalks of Selected Plants. Submitted to Cereal Chem.

2. Molecular Structure of Cereal-Derived Sugars, Sugar-Derived Compounds, and Their Complexes (J. E. Hodge)

- a. Specific Objective: With the similar structures of naturally occurring sweet compounds as models, synthesize compounds to obtain more information on structure-taste relationships.

Progress: Continued syntheses of compounds with chemical structures resembling those of naturally occurring phyllodulcin, one of which was found to be intensely sweet (last report), has given additional information on the functional groups and their stereochemical arrangements required for the induction of sweet taste. The 3-hydroxy-4-methoxyphenyl group of isovanillin was incorporated into flavanone, flavan, and flavan-4-ol structures; also, into dihydrochalcones wherein the phenolic A-ring of flavonoids was replaced by 3-hydroxyfuran and 3-hydroxythiophene radicals. Flavans synthesized by reduction of the carbonyl group of sweet flavanones were not sweet, nor were the furan and thiophene analogs of flavonoid dihydrochalcones sweet. Syntheses of new mono- and di-beta-glucosides of aliphatic alpha-omega glycols of the C<sub>4</sub>, C<sub>6</sub>, and C<sub>8</sub> series were accomplished with syntheses of the corresponding alpha-glucosides still in progress. The beta-glucosides were either bitter or tasteless. It is apparent from this work and investigations by others that the stereochemical and structural requirements for compounds to elicit an intense sweet taste are indeed strict and that the key to the problem is yet to be found.

- b. Specific Objective: Prepare alditols (sugar alcohols obtained by reduction of sugars) and their derivatives to provide new hydrophilic compounds for potential food uses.

Progress: Reduction of maltulose (4-O-alpha-D-glucopyranosyl-D-fructose) produced a mixture of two disaccharide alditols that were not readily separated. Of this pair, the 4-O-alpha-D-glucopyranosyl-D-glucitol (maltitol) is well investigated; but properties of the corresponding D-mannitol, which should be significantly different from those of maltitol, are obscure. Attempted synthesis of the 4-O-alpha-D-glucopyranosyl-D-mannitol showed that a published method for producing alpha-glucosides was faulty; however, a successful modification of this procedure was developed and is accepted for publication. Synthesis of the desired alditol remains to be accomplished. In work not covered by the specific objective, the conformational stereochemistry of a diethylidene acetal of galactitol was determined to advance knowledge of alditol chemistry.

- c. Specific Objective: Identify and characterize compounds in sugar-amine browning products which act as antioxidants in vegetable oils and in corn oil extracted from ammoniated corn kernels.

Progress: The structure of a phenolic compound, C<sub>22</sub>H<sub>20</sub>O<sub>6</sub>, produced by hydrolysis of either C<sub>6</sub> or C<sub>12</sub> reductone-type antioxidants derived from sugar-amine browning reactions, was determined by multistep



synthesis. The C<sub>22</sub> compound proved to be a trihydric di-indanone, a dimeric adduct of two molecules of 3,7-dimethyl-4,5-dihydroxyindenone, all carbons of which are derived from D-glucose through water, amine, and carbon dioxide elimination reactions. The C<sub>22</sub> compound is part of a series of sugar degradation reactions that lead to both alicyclic and heterocyclic compounds, terminating in intractable brown polymers.

Through development of a suitable gas-liquid chromatographic technique, it was demonstrated that both the dialyzable and nondialyzable fractions of glucose-alanine browning reaction melanoidins are better antioxidants than alpha-tocopherol (Vitamin E) when the antioxidants are added in weights equal to that of methyl linoleate in triacetin (synthetic oil) solution. However, decreasing the weight ratio below 1:1 failed to inhibit the oxidation of methyl linoleate.

Study of the pyrolysis products of Amadori compounds containing a secondary amino acid moiety was concluded with the synthesis of two N,N-dimethylfurfurylamines. The prolino- and sarcosino-fructoses form the same cyclized, dehydrated, 6-carbon compounds, among which 4,5-dihydro-5-hydroxymaltol predominates.

#### Publications:

DICK, W. E., JR. Efficiency of Cadmium Carbonate as an Aryl Glycosidation Catalyst: Effect of Lot Variations on Product Compositions. Accepted for publication in Carbohydr. Res.

DICK, W. E., JR. AND J. E. HODGE. An Intensely Sweet Analog of Phyllodulcin: 2-(3-Hydroxy-4-Methoxyphenyl)-1,3-Benzodioxan. J. Agric. Food Chem. 26 (1978):723-725.

MILLS, F. D. Ring Contraction Products from 2,3-Dihydro-3,5-Dihydroxy-6-Methyl-4(H)-Pyran-4-one: 4-Hydroxymethyl-2-Methyl-3(2H)-Furanone and 2,5-Dimethyl-4-Hydroxy-3(2H)-Furanone. J. Agric. Food Chem. 26 (1978):894-898.

RENDLEMAN, J. A., JR. AND J. E. HODGE. Complexes of Carbohydrates with Aluminate Ion. Aldose-Ketose Interconversion on Anion-Exchange Resin (Aluminate and Hydroxide Forms). Accepted for publication in Carbohydr. Res.

SINCLAIR, H. B. Conformation of 1,3:4,6-Di-O-chloroethylidenegalactitol and Its Conversion Into [1(R\*), 4(S\*)]-2,1,3:5,4,6-Di-O-ethanylethylidenegalactitol. Accepted for publication in Carbohydr. Res.

VAN CLEVE, J. W. Reinvestigation of the Preparation of Cholesteryl 2,3,4,6-Tetra-O-benzyl-alpha-D-glucopyranoside. Accepted for publication in Carbohydr. Res.

### Other Reports:

HODGE, J. E. Nonenzymatic Browning: Chemical Reactions that Govern the Acceptance of Seed Products in Foods. Presented in symposium at the Sixth International Cereal and Bread Congress, Winnipeg, Manitoba, Canada, September 16-22, 1978.

HODGE, J. E. Effects of Nonenzymatic Browning on the Nutritional Value of Foods. Presented at the Conference of General Collaborators from North Central Agricultural Experiment Stations, Northern Regional Research Center, Peoria, Illinois, October 23-24, 1978.

### 3. Functional Properties of Proteins in Wheat and Related Grains, Their Flours, and Protein Isolates (J. S. Wall)

- a. Specific Objective: Determine relationships between the structures of proteins of wheat and related cereal grains to their functionality and genetic affinities.

Progress: Protein amino acid sequence analyses were conducted to determine relationships of cereal proteins within complex protein classes and to demonstrate genetic relationships of cereals by homology of corresponding proteins. The identity of high molecular weight gliadin and ethanol-soluble wheat glutenin subunits was confirmed by similar sequences. Amino-terminal sequences of barley and oat prolamines were determined; no sequence homology between them and wheat prolamines was noted. In addition, prolamine fractions of each cereal examined have been found to each consist of proteins very similar to each other in sequence, suggesting that they arose by duplication and mutation of single ancestral genes.

- b. Specific Objective: To employ improved methods of analytical separations of wheat proteins to study genetic relationships between lines of wheat currently under development by breeders.

Progress: An improved polyacrylamide gel electrophoresis method has demonstrated a minimum of 40-50 unique polypeptide subunits in wheat glutenin. Methodology for two-dimensional polyacrylamide gel electrophoresis of gliadin has been adopted. With the use of these along with previous one-dimensional methods for preparation and electrophoresis of gliadin, glutenin, and total proteins from single kernels, samples from breeders and geneticists have been evaluated. An intergeneric Triticum timopheevi x Hordeum bogdanii hybrid was compared to its putative parents; results suggest that an unusual line of one parent was involved in the original cross which suppressed normal intergeneric incompatibility factors. Examination of opaque and vitreous selections of triticale varieties revealed considerable heterogeneity of some samples, but suggested that differences in endosperm type are related to increased amounts of gliadin and lower-molecular weight albumins and globulins in vitreous kernels.



- c. Specific Objective: To assess the role of hydrophobic bonding interactions and lipid binding of wheat gluten proteins.

Progress: Separations of purified native glutenin proteins was carried out by gel filtration column chromatography on agarose in different aqueous solutions including sodium dodecyl sulfate, sodium dodecanoate, concentrated urea, and guanidine hydrochloride at both acid and alkaline pH's. The results demonstrate that glutenin consists of a wide range of different molecular weight proteins. Most of the proteins are disulfide linked; however, glutenin also contains a small fraction of low molecular weight proteins which associate strongly with the other proteins by hydrophobic bonding. The variations in the quantities of different protein fractions and their associations are responsible for the differences in mixing and baking characteristics of flours from different wheat varieties.

- d. Specific Objective: Development of an alkaline wet-milling process for the production of starch and protein concentrate from high protein and high-lysine lines of barleys.

Progress: An alkaline extraction procedure was developed to produce protein concentrate and starch fraction from ground barleys. Protein content of the concentrates varied between 72 and 84%, and the concentrates accounted for 51 to 72% of the total barley proteins. The concentrates had from 2.9 to 5.0 g lysine and 2.1 to 4.2 g total sulfur amino acids per 16 g of nitrogen. All protein concentrates had good hydration capacity.

Publications:

CLUSKEY, J. E., Y. V. WU, AND J. S. WALL. Density Separation of Protein from Oat Flour in Non-aqueous Solvents. *J. Food Sci.* 43 (1978):783-786.

HUEBNER, F. R. AND J. S. WALL. Polysaccharide Interactions with Wheat Proteins and Flour Doughs. *Cereal Chem.*, in press.

SHUEY, W. C., R. D. MANEVAL, J. A. BIETZ, AND O. J. BANASIK. Functional Properties of Various Proteins in Blends with Wheat to Make High-Protein Products. *Cereal Foods World* 23(8) (1978):484 (abstract).

SEXSON, K. R., Y. V. WU, F. R. HUEBNER, AND J. S. WALL. Molecular Weights of Wheat  $\gamma_2$ -,  $\beta_6$ -,  $\alpha_7$ -,  $\alpha_8$ -, and  $\alpha_9$ -gliadins. *Biochim. Biophys. Acta* 532 (1978):279-285.

WALL, J. S. Role of Wheat Proteins in Determining Baking Quality. In *Biochemistry of Cereals*, Edited by D. Laidman and R. G. Wyn Jones, *Phytochemical Society Symposia*, 1978, Academic Press (in press).

WU, Y. V., A. C. STRINGFELLOW, R. A. ANDERSON, K. R. SEXSON, AND J. S. WALL. Triticale for Food Uses. *J. Agric. Food Chem.* 26 (1978):1039-1048.

WU, Y. V. AND A. C. STRINGFELLOW. Protein Concentrate from Air Classification of High-Protein Soft Wheat Flours. J. Food Sci. 44 (1979) (in press).

Other Reports:

BIETZ, J. A. Recent Advances in Isolation and Characterization of Cereal Proteins. Presented at Sixth International Cereal and Bread Congress, Winnipeg, Manitoba, Canada, September 20, 1978.

BIETZ, J. A. AND J. S. WALL. Homology of Cereal Storage Proteins as Revealed by N-Terminal Amino Acid Sequence. Presented at Society for Experimental Biology, Newcastle upon Tyne. April 4-6, 1978.

HUEBNER, F. R. AND Y. V. WU. Functional Properties of Soft Wheat Protein. Presented at the Twenty-Fifth Research Review Conference, Soft Wheat Quality Laboratory, Wooster, Ohio, April 6-7, 1978.

HUEBNER, F. R. AND J. S. WALL. Wheat Glutenin: Effect of Dissociating Agents on Molecular Weight and Composition as Determined by Gel Filtration Chromatography. Presented at the 14th Midwest Regional Meeting, ACS, Fayetteville, Arkansas, October 26-27, 1978.

SEXSON, K. R., Y. V. WU, F. R. HUEBNER, AND J. S. WALL. Molecular Weight Determination of Wheat Glutenin Subunits by Equilibrium Ultracentrifugation. Presented at the 14th Midwest Regional Meeting of the American Chemical Society, Fayetteville, Arkansas, October 26-27, 1978.

WALL, J. S., Genetic Control of Synthesis of Wheat Endosperm Proteins. Presented at Plant Breeding Institute, Cambridge, England, April 17, 1978.

WALL, J. S. Role of Wheat Proteins in Determining Baking Quality, Phytochemical Society Symposium, Bangor, Wales, April 5-8, 1978.

WALL, J. S. Properties of Proteins Contributing to Functionality of Foods in Symposium "Role of Protein and Carbohydrate Properties in Foods." Sixth International Cereal and Bread Congress, Winnipeg, Manitoba, Canada, September 17-23, 1978.

WU, Y. V. AND A. C. STRINGFELLOW. Good Yield of Protein Concentrate by Air Classification of High-Protein Soft Wheat Flours. Presented at 38th Annual Meeting of the Institute of Food Technologists, Dallas, Texas, June 4-7, 1978.

4. Preparation of Protein Products from Corn and Related Cereals (J. S. Wall)

- a. Specific Objective: Compare and trace the genetic relationships and evolutionary development of the numerous races of corn by relating similarities in electrophoretic patterns of zein polypeptides.



Progress: Zein proteins were extracted from endosperm sections of single corn kernels of 12 exotic races and 19 widely grown inbreds. The polypeptides constituting each of these protein extracts gave unique patterns in polyacrylamide gel electrophoresis with pH 3.2 buffer in 8M urea or isoelectric focusing (IEF) pH 5-9 in 8M urea. The IEF procedure gave the highest resolution of zein components and will be employed in genetic studies.

- b. Specific Objective: Determine the nutritional and industrial potential of corn grain bred for high oil content.

Progress: Grain samples from seven experimental hybrids containing 7-10% oil were evaluated for protein composition. The germ portions of these hybrids contained the same levels of protein as normal corn and the protein had the same excellent balance of essential amino acids. However, these varieties contain 33-75% more germ than normal corn. Corn millers would obtain more high-value germ feed as well as oil from these varieties. On a whole grain basis, the experimental hybrids contain up to 15% protein. The increase in protein level was partially from increased germ size but mostly from increased endosperm protein levels. Lysine was the only seriously deficient amino acid. The experimental varieties contained, with one exception, 2.4-2.5% lysine expressed as a percent of protein and 0.30-0.39% expressed on a whole grain basis compared to values of 3.0% and 0.29%, respectively, for normal corn. Differences in fiber and ash values in these experimental varieties were not nutritionally significant.

- c. Specific Objective: Isolate and determine the primary amino acid sequences of individual zein polypeptides to relate structures to properties and genetic variability.

Progress: Although individual zein polypeptides are not yet available for sequence analysis, a 33-residue amino-terminal sequence of whole zein from two normal and one opaque-2 corn line has been completed. The high degree of homology noted among zein polypeptides suggests a close relationship not only within each of the two major molecular weight groups, but also between them; apparently the larger type of zein has a carboxyl-terminal extension or internal insertion of about 20 amino acid residues. Prolamines from sorghum, teosinte, and tripsacum showed homology in the N-terminal portion to zein.

- d. Specific Objective: Study the composition of lipoproteins and glycoproteins present in zein and glutelin preparations, since these components may be derived from membrane and cell wall fractions.

Progress: Yellow lipoprotein present in zein preparations accompanied beta zein upon fractionation of zein with 95% ethanol. Pigment associated with beta zein was mainly the carotenoid zeatin. Alpha and beta zein differed slightly in amino acid composition. Polyacrylamide gel electrophoresis in sodium dodecyl sulfate demonstrated that native beta zein was high molecular weight (MW) and contained no 22,000 MW component. Upon reduction it had a fast moving subunit not

present in reduced alpha zein. Alpha zein separated into 4 components differing in MW on gel filtration chromatography in 8M urea.

A glycoprotein isolated from corn endosperm after extracting protein and digesting starch contained 20% protein. The 80% carbohydrate consisted of 9.9% cellulose and hemicellulose containing 16.7% arabinose, 16.4% xylose, 2.4% mannose, 2.7% galactose, and 20.6% glucose. About 67% of the glycoprotein dissolved after digestion with xylanases. Endoxylanase degraded the glycoprotein more completely than exoxylanases indicating presence of considerable internal xylose.

#### Publications:

BIETZ, J. A., J. W. PAULIS, AND J. S. WALL. Zein Subunit Homology Revealed through Amino-terminal Sequence Analysis. Cereal Chem., in press.

NIELSEN, H. C., J. S. WALL, AND G. E. INGLET. Protein-and Fiber-Containing Flour from Wet-Mill Corn Germ with Potential Food Use. Cereal Chem. (in press).

PAULIS, J. W. AND J. S. WALL. Distribution and Electrophoretic Properties of Alcohol-Soluble Proteins in Normal and High-Lysine Sorghums. Sorghum Newsl. 21 (1978).

PAULIS, J. W. AND J. S. WALL. Distribution and Electrophoretic Properties of Alcohol-Soluble Proteins in Normal and High-Lysine Sorghums. Cereal Chem. (in press).

PAULIS, J. W., J. S. WALL, AND J. SANDERSON. Origin of High Methionine Content in Sugary-1 Corn Endosperm. Cereal Chem. 55(5) (1978):705-712.

SANDERSON, J. E., J. W. PAULIS, F. N. PORCUNA, AND J. S. WALL. Sweet Corn: Varietal and Developmental Differences in Amino Acid Content and Composition of Grain. Cereal Chem., in press.

WU, Y. V. Protein Concentrate from Normal and High-Lysine Sorghums: Preparation, Composition, and Properties. J. Agric. Food Chem. 26 (1978):305-309.

#### Other Reports:

NIELSEN, H. C., J. S. WALL, J. E. SANDERSON, AND G. E. INGLET. High-Oil Corn Selections That Contain Increased Amounts of Protein: Variations in Nutritional Quality of Protein. Presented at American Society of Agronomy Meeting, Chicago, Illinois, December 3-8, 1978.

PAULIS, J. W. AND J. S. WALL. Distribution and Electrophoretic Properties of Alcohol-Soluble Proteins in Normal and High-Lysine Sorghums. Presented at American Society of Agronomy Meeting, Chicago, Illinois, December 3-8, 1978.



WALL, J. S. Chemical Basis for Genetic Variation in Corn Proteins. Presented before Plant Genetics Department Seminar, University of Missouri, Columbia, Missouri, September 27, 1978.

5. Interactions of Food Carbohydrates (J. E. Hodge)

- a. Specific Objective: Increase the nutritive value of bakery goods by incorporating high-protein flours and dietary fiber in combination with food gum-starch systems in place of wheat gluten.

Progress: Addition of polysaccharide gums (guar, xanthan) to soft wheat cake flour induced improved shear-thinning of the mixture, it also permitted use of less shortening for the plasticization needed to disperse gas bubbles during chemical leavening and baking. The gel-forming gums were shown to interact with soluble starch of the flour to provide (a) a binding layer for added protein (soy protein isolate in place of egg and milk proteins) and (b) an efficient moisture sink for control of thermal setting (redistribution of water within the dough). The soluble starch-gum gel replaced swollen starch granules as a moisture sink; hence, a high sugar concentration was not needed to inhibit full gelatinization of the starch. Low-sugar, low-fat cakes were baked with acceptable volume and texture. This work has been completed. Study of these interactions of baking ingredients was undertaken to exemplify preparation of baked goods with significantly lower caloric values and with retention of crumb cohesiveness and protein content.

- b. Specific Objective: Determine the effect of food gums on the properties of gelatinized wheat starch and investigate physical interactions between starch granules and food gums.

Progress: Further studies were undertaken to define the interactions between hydrocolloid gums (neutral guar, ionic cellulose, ionic xanthan) and starch granules immediately before and during gelatinization in hot water. A procedure was developed for determining the electrostatic charge on starch granules. It involved measurement of the voltage applied to inhibit electrophoretic migration of a granule in an inert suspension medium, using the recently developed zeta potential instrument. Granule charge varied with starch type, added gum type, amylose content of the starch, extent of hydration, and granule density. With polarized laser light at a single wavelength, changes in molecular organization within the granules during swelling were observed directly, thereby circumventing the usual embedding and sectioning procedures. Xanthan was shown to be superior to guar and ionic cellulose gum in promoting diffusion of starch from granules in hot water, with less retrogradation of the pastes on cooling, and formation of more stable gels. Gelatinization differences and complexing between starch molecules and the gums were defined by scanning electron microscopy, synergism in viscosities of the soluble starch fraction at increasing shear rates, and iodine uptake of the solubles. These fundamental studies of starch gelatinization in the presence of gums are expected to promote reduction of bread staling and textural improvements in starch-based foods.

- c. Specific Objective: Develop analytical procedures for determining the sugars and uronic acids of dietary fiber carbohydrates.

Progress: A gas-liquid chromatographic analysis was developed for determining gluconic and glucuronic acid residues in the presence of the other sugars of hemicellulose hydrolyzates. The analysis is based on a differential determination of sugar residues through alditol acetate and aldonitrile derivatives. The hexonic and hexuronic acids are retained in the alditol acetate method but are destroyed in the aldonitrile method.

- d. Specific Objective: Determine the chelating and ion-exchange properties of wheat bran with respect to polyvalent cations of nutritionally important minerals.

Progress: A colorimetric procedure using the metal-ion complexing agent, tetramethylmurexide, was developed for determining residual calcium and zinc ions in dilute aqueous solutions of their salts after uptake of these ions by wheat bran, separated bran components, and constituents of gastric fluids. A standardized white wheat bran and its aqueous extract were shown to have a large capacity for binding calcium ion. Defatted, cooked, and acidified bran bound even larger amounts. Dextrose, starches, amylose, amylopectin, pure cellulose, 56% methylated pectin, glycine, serum albumin, saliva, and gastric mucin bound little or no calcium ion from dilute, neutral, aqueous solutions. Both polygalacturonic acid and phytate bound calcium ion strongly; however, binding by phytate was highly sensitive to pH variations (2.5 times more calcium bound at pH 6.6 than at pH 5.3), whereas bran binding capacity was relatively insensitive to pH variation in the range 5.7-7.5 (6% less calcium bound at pH 6.8 than at pH 5.7). Wheat bran was shown to be a strong buffering agent for hydrochloric acid. The results indicate that water-soluble constituents of bran (e.g., acidic hemicelluloses) are the principal calcium-binding agents, not phytates. This study is designed to contribute to knowledge of the physiological effects of dietary fiber.

- e. Specific Objective: Investigate interactions between food-grade gums and amino acids, peptides, and proteins.

Progress: Although the viscosities of pastes of ionic gums were increased significantly by the addition of basic amino acids and cysteine, no clear demonstration that these interactions inhibited nonenzymatic browning reactions of the basic amino acids with reducing sugars could be established.

Publications:

RENDLEMAN, J. A., JR. Metal-Polysaccharide Complexes. Part I and Part II. Food Chem. (London) 3 (1978):47-79, 127-162.



### Other Reports:

CHRISTIANSON, D. D., H. W. GARDNER, AND K. A. WARNER. Ingredient Engineering of Chemically Leavened Baked Goods: Development of Starch or Soft Wheat Flour Polysaccharide-Gum Systems. Presented at 38th Annual Meeting, Institute of Food Technologists, Dallas, Texas, June 4-7, 1978.

CHRISTIANSON, D. D., K. A. WARNER, H. W. GARDNER, AND M. K. KEEL. Plant Protein and Fiber Fortification of Starch-Gum Base Breads Without Gluten. Presented at 38th Annual Meeting, Institute of Food Technologists, Dallas, Texas, June 4-7, 1978.

CHRISTIANSON, D. D. Starch-Hydrocolloid Complexes and Their Effect on Rheology, Texture, and Storage of Starchy Foods. Presented in Seminar, Hercules, Inc., Wilmington, Delaware, August 16, 1978.

LEHRFELD, J. New Spectrophotometric Methods for the Analysis of Gluconolactone. Presented at ACS Regional Meeting, Indianapolis, Indiana, May 24, 1978.

LEHRFELD, J. Hemicellulose Analysis by GLC: A Comparison Between the Alditol Acetate and the Aldononitrile Methods. Presented at 178th National Meeting, American Chemical Society, Miami Beach, Florida, September 11-17, 1978.

### 6. Corn Starches--Physical Characteristics and Biological Digestibilities (G. E. Inglett)

- a. Specific Objective: To continue fractionation procedures of different kinds of starch (ex: those of varying amylose content) by various methods, both known and newly developed; to characterize the resulting various sized starch granules as to their composition and properties; and to investigate the microscopic properties of starches of corn endosperm mutants.

Progress: Four kinds of corn starch--normal dent, waxy, and amylomaize, Classes V and VII--were fractionated into multiple size ranges by a particle density separation in aqueous medium. Selected fractions were observed under light microscopy to determine the efficiency of separation procedure. Photomicrographs were taken and visual measurements of granule size dimensions were made. Characterization of the separated sized granules was extended by means of iodine binding studies at 2 and 20 C. A modified approach in the potentiometric method of iodine binding was developed. Apparent amylose content, as measured by iodine binding capacity, was found to vary inversely with granule size in the amylomaize starches. No significant variation was observed in normal dent corn starch. Spectrophotometric characterization of the unfractionated starches and the fractional cuts was carried out concomitantly.

Mutant gene starch (single mutant) was extracted with difficulty from single kernels. A steeping evaluation series for single kernel

starch extraction was completed. Fixation and infiltration with resin of normal and mutant endosperms were done for photomicrographic studies. Normal corn and mutant selections were planted in test garden plot to increase the research corn supply. In situ starch studies and cellular structure examination of mutants are underway.

#### Reports:

CLUSKEY, J. E. Biodigestibility of Corn Starches. 19th Annual Corn Dry Milling Conference, Northern Regional Research Center, May 31-June 1, 1978, Peoria, Illinois.

CLUSKEY, J. E., U. KHOO, C. A. KNUTSON, AND G. E. INGLETT. Starches of Various Corn Genotypes and Their Nutritional and Utilization Aspects. 28th Annual Conference, National Sweet Corn Breeders Association, December 11-12, 1978, Chicago, Illinois.

### B. CHEMICAL RESIDUES AND ADDITIVES IN FOOD AND FEED

#### 1. Environmental Contaminants in Cereals and Processed Cereal Foods (C. W. Blessin)

- a. Specific Objective: Complete research on relative abundance of heavy metals in selected food crops grown on normal soil types with mineral and organic fertilizers.

Progress: Selected crops, grown in different soil materials amended with anaerobically digested sludge and a commercial fertilizer, were analyzed for content of lead, mercury, cadmium, and zinc. These included edible parts of lettuce, tomatoes, onions, radishes, and wheat grain and also wheat plants. Movement of the four metals from the soil materials was manifested in lettuce and concentration of the metals varied considerably. On the other hand, tomatoes, even when grown in widely diverse soil materials, did not accumulate elevated levels of metals. Furthermore, it was not possible to relate soil medium to metal contents of tomatoes.

Total wheat plants and the associated grain were also analyzed to evaluate the translocation of metals. To relate the accumulation of metals by the total plant to those occurring in the grain, grain to plant metal concentration ratios were calculated. For all soil treatments, mean ratio data demonstrated that the grain accumulated smaller quantities of lead (0.033) and mercury (0.068) than the total plant. However, for cadmium, the mean ratio (0.423) showed that concentrations in the grain more nearly approach those in the plant. By contrast, zinc was accumulated at higher concentration ratios in the grain (1.32) than in the plant.

Finally, the metal contents of the five edible crops (tomatoes, lettuce, onions, radishes, and wheat grain) were compared. The mean



lead concentration ratios for the crops, grown in the different soil materials, ranged from a low of 1.00 for tomatoes to a high of 93.94 for lettuce. Likewise, for cadmium and mercury, respectively, the same comparison showed lettuce concentrations 19.70 and 45.81 times greater than tomatoes. However, the highest zinc concentrations were found in the grain (36.83 times greater than in the tomatoes). The lowest metal accumulations were associated with black top soil, brown loam, and clay.

- b. Specific Objective: Initiate research to determine the effect of ambient ozone generated by air pollution on composition, nutritional quality, and safety of corn grain.

Progress: Ozone ( $O_3$ ) is the most destructive photochemical oxidant in air pollution affecting agriculture in the United States. Three corn hybrids--Coker 16; FR 632 X FR 619; and H 95 X FR 64A--were each grown at three experimental levels of atmospheric ozone--0.02 ppm (carbon-filtered air), 0.06 ppm (ambient air), and 0.15 ppm (ozone-enriched air). Plant height, weight of stover, yield of grain, and weight of individual kernels were less at 0.15 ppm  $O_3$  than at the other two concentrations. Data on the harvested grain, which included proximate analyses (ash, fiber, fat, and protein), total starch, and amylose content of the starch, were similar for the three levels of ozone. Amino acid composition of the protein in the grains also was not affected by ozone. However, mineral content of the grain did vary due to the effect of ozone concentration.

Publications:

BLESSIN, C. W., W. L. DEATHERAGE, J. F. CAVINS, W. J. GARCIA, AND G. E. INGLETT. Preparation and Properties of Defatted Flours from Dry-milled Yellow, White and High-lysine Corn Germ. Cereal Chem. (in press).

BLESSIN, C. W. AND W. J. GARCIA. Heavy Metals in the Food Chain by Translocation to Crops Grown on Sludge-treated Strip Mine Land. Pennsylvania State University Press (in press).

Other Reports:

BLESSIN, C. W. Corn Germ Flour--A New Food Ingredient. Presented at the Land, Food and You Conference, Illinois Central College, East Peoria, Illinois, March 28 and 29, 1978.

BLESSIN, C. W. Dry-milled Germ Flours from Yellow, White and High-lysine Corn. Presented at the Annual Corn Dry Milling Conference, NRRC, May 31 and June 1, 1978.

GARCIA, W. J. Corn Products Endogenously Labeled with  $^{65}Zn$  for Bioavailability Studies. Presented at the Annual Corn Dry Milling Conference, NRRC, May 31 and June 1, 1978.

## C. FOOD COMPOSITION AND IMPROVEMENT

### 1. Composition and Properties of Cereal Grain Fibers for Foods (F. R. Dintzis)

- a. Specific Objective: Examination of factors influencing ferric and ferrous iron binding of wheat bran will continue in order to determine methods to reduce potential loss of mineral bioavailability in human diets.

Progress: Binding of ferric iron under simulated human gastric solution conditions has been measured for a variety of wheat brans.

- b. Specific Objective: Cooperation with SEA-AR's Human Nutrition Research Laboratory, Grand Forks, ND, will continue as dietary roughages will be supplied and examined in order to correlate effects of dietary fiber type and composition with diet and physiological effects upon humans.

Progress: We continue to supply desired fiber sources to HNRL as required and have made available an initial batch of demineralized bran. Remnants of brans have been retrieved from fecal matter and composition compared with that of starting material.

- c. Specific Objective: Develop methodology for determining total bile acid concentration in fecal samples. Identify and quantitate prominent bile acids in human fecal samples obtained from subjects fed various dietary fiber.

Progress: Quantitation of bile acids in a series of fecal samples from subjects fed basal, soft winter wheat, bran, hard red spring wheat, corn bran, and soybean hull diets has been completed. No obvious correlation between total bile acid excretion or individual fecal bile acid content and dietary fiber was discovered. In general, large variations in the total bile acid data was noted. This large variation could not be traced to errors in the analytical methodology, although the total bile acid content found in these samples was about 19% higher than values reported in the literature.

#### Publications:

DINTZIS, F. R. AND R. TOBIN. Light Scattering and Specific Refraction Increment Behavior of Amylose and Dextran in Methyl Sulfoxide-water. Carbohydr. Res. 66 (1978):71-83.

DINTZIS, F. R., L. M. LEGG, W. L. DEATHERAGE, F. L. BAKER, G. E. INGLETT, R. A. JACOB, S. J. RECK, J. M. MUÑOZ, L. M. KLEVAY, AND H. H. SANDSTEAD. Human Gastrointestinal Action upon Wheat, Corn, and Soy Hull Bran--Preliminary Findings. Cereal Chem. (in press).

DINTZIS, F. R., J. B. MCBRIEN, F. L. BAKER, G. E. INGLETT, R. A. JACOB, J. M. MUÑOZ, L. M. KLEVAY, H. H. SANDSTEAD, AND W. C. SHUEY. Human Gastrointestinal Action upon a Hard Red Wheat Bran. Symposium



Proceedings on "Dietary Fibers: Chemistry and Nutrition," American Chemical Society Meeting, Miami Beach, Florida, September 11-12, 1978, Edited by G. E. Inglett and I. Falkehag, Academic Press (in press).

SANDSTEAD, H. H., L. M. KLEVAY, R. A. JACOB, J. M. MUÑOZ, G. M. LOGAN, JR., S. J. RECK, F. R. DINTZIS, G. E. INGLETT, AND W. C. SHUEY. Effects of Dietary Fiber and Protein Levels on Mineral Element Metabolism. Symposium Proceedings on "Dietary Fibers: Chemistry and Nutrition," American Chemical Society Meeting, Miami Beach, Florida, September 11-12, 1978, Edited by G. E. Inglett and I. Falkehag, Academic Press (in press).

#### Other Reports:

DINTZIS, F. R., J. B. MCBRIEN, F. L. BAKER, G. E. INGLETT, R. A. JACOB, J. M. MUÑOZ, L. M. KLEVAY, H. H. SANDSTEAD, AND W. C. SHUEY. Human Gastrointestinal Action upon a Hard Red Wheat Bran. Presented at symposium, American Chemical Society meeting, Miami Beach, Florida, September 12, 1978.

SANDSTEAD, H. H., L. M. KLEVAY, R. A. JACOB, J. M. MUNOZ, G. M. LOGAN, JR., S. J. RECK, F. R. DINTZIS, G. E. INGLETT, AND W. C. SHUEY. Effects of Dietary Fiber and Protein Levels on Mineral Element Metabolism. Presented at symposium, American Chemical Society meeting, Miami Beach, Florida, September 11, 1978.

## ENGINEERING AND DEVELOPMENT LABORATORY

E. B. Bagley, Chief

Research Leaders: R. A. Anderson, R. J. Bothast, G. E. Hamerstrand,  
and G. C. Mustakas

### A. TECHNOLOGIES FOR FOOD AND FEED USES OF FIELD CROPS

1. Effects of Isoelectric Precipitation and of Heat on Soybean Proteins  
(K. L. Moulton)

See Oilseed Crops Laboratory, B.1.

2. Parching Process for Wild Rice (R. A. Anderson)

- a. Specific Objective: Develop new methods of parching wild rice for the purpose of increasing process efficiency.

Progress: A continuous process for parching wild rice was developed as an alternative procedure for the batch-type systems now being used in industry. The new method is essentially a two-stage one, in which a continuous interrupted flight mixer-conveyor is used to first, steam the grain and, second, to dry the grain. Kernel strength (KS) defined as weight of whole kernel divided by weight of whole and broken kernels, was designated as an indicator of breakage. In using the new procedure, KS values of finished rice ranged from 0.81 to 0.87 as compared to 0.8 obtained from the average of 6 commercial rice samples.

- b. Specific Objective: Establish uniform quality parameters for wild rice.

Progress: Quality characteristics of finished rice from the new parching system are similar to commercial wild rice, with the exception of the absence of a slight toasted flavor generally present in the commercial rice. This flavor can be simply added by a slight toast in a continuous roaster or drier. Steaming of the green wild rice appears to have beneficial effects to subsequent rice processing in reducing breakage and eliminating white centers. Compositional analysis of hulls and other offal from the processing of wild rice shows about 12% protein, of which 4.7 to 5.0% is lysine and 3.1-3.7% represents the sulfur-amino acids. Crude fiber content is 21 to 25%.

Publications:

ANDERSON, R. A., C. VOJNOVICH, L. L. NAVICKIS, AND E. B. BAGLEY.  
Parching Studies on Wild Rice. Cereal Chem. (accepted for publication November 3, 1978).



ANDERSON, R. A. Wild Rice: Its History, Current Production, Use. Rice J. 81, No. 7 (1978):34-38.

NAVICKIS, L. L. AND R. A. ANDERSON. Composition of a By-Product of Wild Rice Processing. Cereal Chem. 55 (1978):544-546.

Other Reports:

ANDERSON, R. A. Progress Report of Wild Rice Research at NRRC. Presented at annual meeting of Wild Rice Growers Association, Bemidji, Minnesota, January 27-28, 1978. Also distributed as a handout.

ANDERSON, R. A., L. L. NAVICKIS, K. A. WARNER, C. VOJNOVICH, AND E. B. BAGLEY. Quality Characteristics of Processed Wild Rice. Presented at the Sixth International Cereal and Bread Congress, Winnipeg, Manitoba, September 16-22, 1978. Submitted to Cereal Chemistry for publication.

3. Conversion of Cereals and/or Their Fractions and Components to New or Improved Foods (R. A. Anderson and G. N. Bookwalter)

- a. Specific Objective: Continue development and evaluation of new cereal food products with improved nutritional quality.

Progress: Heat treatment of some food blends is undertaken to eliminate contamination by salmonella with minimum heat treatment and to investigate the effect of this treatment on the nutritive value of the blends. Enriched blends of corn meal, soy flour, and nonfat dry milk (CSM) were adjusted to 7 and 10% moisture and inoculated with  $10^3$  cells/g of Salmonella anatum and Salmonella senftenberg.

The blends were stored at -18 to 60 C and tested for salmonellae and nutritional changes. S. senftenberg was more heat resistant than S. anatum. At 43, 49, 54, and 60 C, S. senftenberg was killed after 13, 10, 8, and 6 days, respectively, independent of product moisture. Smaller nutritional losses were observed at 7% than at 10% moisture. Changes in PER values, vitamins A and C and available lysine were minimal at 49 C. At 54 and 60 C nutrient, losses were excessive at 10% moisture.

- b. Specific Objective: Determine milling characteristics of corn dried in energy conserving ways.

Progress: Six sublots of picker-shelled, high-moisture corn were treated with ammonia, sulfur dioxide, methyl-bis-propionate (MBP) and formalin (37% formaldehyde) and ambient air dried as alternatives to energy intensive high-temperature drying. Dry milling of the treated corn samples indicated that only MBP treated corn gave significantly ( $P = 0.05$ ) lower recoveries of prime products (low fat), with yields being 8% lower than the control. Fat content of prime products from all treatments was similar to that of control. Organoleptic evaluation

of second and third break grit products from ammonia and MBP treated corn scored the same as control, while grits from sulfur dioxide and formalin treated corn scored significantly different.

Preliminary indications are that the most energy sensitive operation in the corn dry milling industry, having potential for improvement is the tempering operation. Addition of water to condition the grain results in ultimately having to dry the milled products prior to further processing or storage.

#### Publications:

BOOKWALTER, G. N. AND R. A. ANDERSON. Improving Nutritional Properties of Sorghum by Fortification with Oilseed Proteins. Proceedings 10th Biannual Grain Sorghum Research and Utilization Conference, Wichita, Kansas, March 2-4, 1977, p. 67.

BOOKWALTER, G. N., K. A. WARNER, R. A. ANDERSON, AND E. B. BAGLEY. Cornmeal/Peanut Flour Blends and Their Characteristics. J. Food Sci. 43 (1978):1116-1120.

BOOKWALTER, G. N., K. A. WARNER, R. A. ANDERSON, AND E. B. BAGLEY. Peanut-fortified Food Blends. J. Food Sci. (accepted for publication).

STRINGFELLOW, A. C., L. H. BURBRIDGE, V. F. PFEIFER, AND O. L. BREKKE. Milling and Air-Classification of Alkali-Peeled Wheats. Milling, Feed and Fertilizer CLXI, No. 2 (1978):35-39.

WU, Y. V., A. C. STRINGFELLOW, R. A. ANDERSON, K. R. SEXSON, AND J. S. WALL. Triticale for Food Uses. J. Agric. Food Chem. 26 (1978): 1039-1048.

#### 4. Engineering Factors Affecting Design and Operation of Immobilized Enzyme Processes for Foods and Feeds (E. B. Bagley)

- a. Specific Objective: Refine model of kinetics of soluble batch enzyme reaction system and compare with continuous reactor behavior under (b) below.

Progress: In the last report it was shown that the following reactions occur when raffinose is treated with a crude enzyme mixture of alpha-galactosidase and invertase.

- |    |           |                            |   |                        |
|----|-----------|----------------------------|---|------------------------|
| 1) | Raffinose | <u>alpha-galactosidase</u> | → | sucrose and galactose  |
| 2) | Sucrose   | <u>Invertase</u>           | → | glucose and fructose   |
| 3) | Raffinose | <u>Invertase</u>           | → | melibiose and fructose |
| 4) | Melibiose | <u>alpha-galactosidase</u> | → | glucose and galactose  |

In refining the batch kinetics, it was found that at raffinose concentrations greater than 1.5% an unknown triose appeared. This triose



has been only partially characterized. In addition to the raffinose kinetics [Eq. (1) and (3)], the conversion of sucrose and melibiose [Eq. (2) and (4)], have been examined separately, both in terms of initial velocities (Hines, Hofstee and Lineweaver-Burk plots) and the integrated procedure of Levenspiel. Reasonable agreement was found, indicating no inhibition effects. The reaction constants are being employed in a model for the batch process (Eqs. 1 to 4) and also compared with the kinetics developed for the continuous hollow fiber enzyme reactor.

To study reaction (3) it was necessary to prepare essentially pure invertase. Isolation of the invertase was not possible, but modifications of the cultural conditions were developed that resulted in an enzyme mixture with an alpha-galactosidase to invertase ratio of 0.02 as opposed to 0.40. This essentially pure invertase was then used to evaluate the kinetics of Eq. (3).

- b. Specific Objective: Model a continuous feed hollow fiber reactor (HFER) for the enzyme system, continuing the investigation of factors which limit the application of this reactor system to certain soy food products.

Progress: Reaction kinetics have been followed in the HFER using the mixed crude enzyme system alpha-galactosidase/invertase with raffinose, sucrose, and melibiose separately as substrates. The approximate mathematical solution to the operation of the HFER reported previously, while useful, proved inadequate to explain fully the observed effects of substrate concentration. An exact mathematical solution is being carried out in cooperation with SEA's Data Application Systems Division and their contractor Control Data Corporation. The preliminary programming work is completed and the analysis can now be applied to the experimental data available.

- c. Specific Objective: Compare alpha-galactosidase and invertase immobilized in hollow fiber enzyme reactors with the same enzymes immobilized by other methods, to have comparative study of alternate approaches to the hollow fiber system.

Progress: Sephadex and calcium phosphate gel were among supports used in entrapment and adsorption studies. The two enzymes have been absorbed in calcium phosphate gel and reactions were carried out with melibiose, sucrose, and raffinose as substrates. Kinetics of the systems are being evaluated. It was found, in addition, that alpha-galactosidase and invertase were both removed from the gel on successive elutions of increasing ionic strength of  $\text{KH}_2\text{PO}_4$  at pH 5.0, without affecting the enzyme activity.

5. Basic Engineering Studies on Preparation of Soy Foods for the Export Market (G. C. Mustakas)

- a. Specific Objective: To investigate basic engineering and rheological aspects of extrusions in relation to soy protein quality in foods for the export market.

Progress: An extruder with capillary dies of a variety of dimensions was used to investigate the rheological properties of defatted soy dough. In previous studies at low temperature, plug flow occurred. However, in this study at higher temperatures and moisture of food extruders, flow became viscoelastic as expected. Theories developed for extrusion of thermoplastics are being used to evaluate data collected at various shear rates, temperatures, and die sizes. A simple hand texturizer with instrumentation was built and preliminary studies have shown that the hand texturizer can be used to cook and texturize defatted and full-fat soy doughs. A file of recent papers on food extrusion and rheology was assembled. This included use of the current awareness computer retrieval system for selecting applicable current literature.

- b. Specific Objectives: Optimize the membrane process for treating soybean whey by reverse osmosis to develop a commercial plant design.

Progress: Based on experimental results of using reverse osmosis to reduce environmental pollution from soy whey produced in preparing lipid-protein concentrate, computer simulation studies have been carried out to optimize the whey treatment. Assuming the processing of 100,000 gallons of soybean whey, pumping energy and power costs combined with membrane life and replacement costs were incorporated into the original mathematical model of the process to provide a basis for the cost data. Initial biological demand for 8000 ppm could be reduced by 94% at a cost of \$5.45 per 1000 gallons.

- c. Specific Objective: Establish relations between soybean processing variables and the nutritive value of soybean meal in the diet of the chick. (Cooperative research with University of Maryland Animal Science Department.)

Progress: The soybean solvent extraction pilot plant at NRRC has been completely renovated. An experimental desolventizer-toaster was designed and built that is capable of closely simulating the commercial toasting process as carried out in the large vertical compartmented designs. The treatment of the soybeans is being carefully monitored to establish the critical variables and to correlate them with bioavailability of amino acids in later chick studies. A number of shakedown runs have been completed in preparation for the processing of two large cultivars of soybeans (3 tons) grown in Maryland during the last season.

- e. Specific Objective: Cooperate with SEA-AR's Western Regional Research Center (WRRC) and the Agency for International Development to assist with developing a soy-fortification program of wheat products in Bolivia.

Progress: During August 1979, an NRRC engineer visited Bolivia at the request of WRRC and AID to assist in the conversion of a soybean extraction plant to one having the capability of producing edible-grade soybean flour. The process began with the inspection of soybean



quality and storage and continued on with all the processing steps and sanitary aspects being carefully reviewed. A full report of existing facilities and recommended new equipment and changes was made and presented to AID-WRRC.

- f. Specific Objective: Investigate the bioavailability of zinc and other minerals in soy protein diets. (Cooperative project with the University of Illinois.)

Progress: Two forms of acid-precipitated soy isolates were produced and sent to the University of Illinois, Department of Food Science, for animal feeding tests. One sample was produced in the acid form and the other as the neutralized soy isolate. The products were fed to young rats to determine (1) the bioavailability of endogenous minerals from the products and (2) the availability of minerals added to diets containing these products. The results to date suggest that both zinc and magnesium are highly available from the acid product. There appears to be a decreased availability of endogenous zinc but not magnesium from the neutralized soy concentrate.

Publications:

BAKER, E. C., G. C. MUSTAKAS, M. D. MOOSEMILLER, AND E. B. BAGLEY. Water and Solute Transport Across Cellulose Acetone Membranes in the Treatment of Soybean Whey by Reverse Osmosis. J. Appl. Polym. Sci. (accepted for publication October 5, 1978).

BAKER, E. C., M. D. MOOSEMILLER, AND G. C. MUSTAKAS. Computer Optimization of Reverse Osmosis Processing of Soy Whey with Cellulose Acetate Modules, J. Appl. Polym. Sci. (received by Journal, October 30, 1978).

JASBERG, B. K., G. C. MUSTAKAS, AND E. B. BAGLEY. Extrusion of Defatted Soy Flakes--A Model of a Plug Flow Process. Accepted for publication by Transactions of the Society of Rheology, October 25, 1978.

MUSTAKAS, G. C. AND K. D. CARLSON. Simple Model for Determining Economic Feasibility of Processing New Oilseeds. J. Am. Oil Chem. Soc. 56 (1979):29-32.

WANG, H. L., G. C. MUSTAKAS, W. J. WOLF, L. C. WANG, C. W. HESSELTINE, AND E. B. BAGLEY. Soybeans as Human Food--Unprocessed and Simply Processed, USDA, Utilization Research Report No. 5, January 1979.

Other Reports:

BAKER, E. C., G. C. MUSTAKAS, AND K. A. WARNER. Extraction of Defatted Soybean Flours and Flakes with Aqueous Alcohols: Evaluation of Flavor and Functional Properties. Presented at 176th National American Chemical Society Meeting, Miami Beach, Florida, September 10-15, 1978.

BAKER, E. C. Extraction of Defatted Soybean Flours and Flakes with Aqueous Alcohols: Evaluation of Flavor and Functional Properties. Presented at Conference of General Collaborators from N.C. Agricultural Experiment Station at Northern Regional Research Center, October 23, 1978.

## B. TECHNOLOGIES FOR MARKETING OF FIELD CROPS

### 1. Microbial Spoilage in Corn and Grain Sorghum (E. B. Bagley)

- a. Specific Objective: Explore ambient air drying of high-moisture corn using extenders such as ammonia and sulfur dioxide in small and large-scale tests as well as develop information for Environmental Protection Agency approval for the process.

Progress: Sulfur dioxide ( $\text{SO}_2$ ) was shown to be an effective mycostat. In tests carried out on 23% moisture corn; intermittent ("trickle") application of 0.27%  $\text{SO}_2$  during ambient air drying over a period of 161 days resulted in No. 1 grade corn. A control dried over same period with no added  $\text{SO}_2$  graded No. 5 because of microbial damage. Dry milling of corn dried using either  $\text{SO}_2$  or  $\text{NH}_3$  as mycostatic agents was satisfactory. An organoleptic evaluation of grits from the milling indicated no flavor or odor alterations due to  $\text{NH}_3$ , but  $\text{SO}_2$  treatment does have some deleterious effects though not enough to preclude use of  $\text{SO}_2$  in drying material for use as animal feed. Fatty acid composition of the oil was not affected by the  $\text{SO}_2$  treatment, but there was about a 30% decrease in thiamine. A petition was submitted to EPA for the approval of the NRRC "trickle ammonia process," and the petition was published in the Federal Register. A response to comments is being prepared. A petition on the use of  $\text{SO}_2$  is being prepared.

- b. Specific Objective: Investigate solid substrate fermentation as a process for improving the nutritional value of grain and grain residues and producing metabolites.

Progress: Although ammonia-treated corn fermented with Scopulariopsis brevicaulis shows increased protein content over unfermented corn, rat feeding trials showed that the protein was nutritionally unavailable. For rats the Protein Efficiency Ratio (PER) dropped from 1.3 to 0.9 as a result of the treatment with Scopulariopsis. With chick feeding trials, the Scopulariopsis treated corn gave the same results as did the control corn, but no better. Consequently, work in this area has been stopped.

Solid substrate fermentations are of considerable current interest. Such fermentations are difficult to study in static systems and consequently a laboratory scale rotating reactor used in tissue culture, the Rollacell, was used to investigate the fermentation of Aspergillus awamori NRRL 4869 on 50% moisture wheat bran. Parameters



of solids content, rotation rate, airflow rates, solids consumption, and pH were related to the enzymes formed, alpha-galactosidase and invertase. The reproducibility of results from the Rollacell were much better than from the static control fermentation flasks. Both the yield of the two enzymes and their ratios could be varied and controlled. The enzymes were subsequently used in the research on the hollow-fiber enzyme reactor. In particular, the ability to control the ratio of alpha-galactosidase to invertase was critical in elaborating some details of the kinetics of the conversion of raffinose to sugars as reported under Engineering and Development Laboratory (ED), A.4.

- c. Specific Objective: Solve emergency problems associated with microbial deterioration and contamination in food and grain.

Progress: Because little information is available on the potential human and animal health hazards associated with the handling of aflatoxin-contaminated corn, preliminary experiments were conducted to determine the levels of airborne Aspergillus flavus spores and aflatoxin during the transfer and cleaning of corn containing approximately 380 ppb aflatoxin. Adjacent to the grain augers and cleaner at a field site, the A. flavus spore count ranged from  $2.5 \times 10^5$  to  $1.4 \times 10^2/\text{m}^3$  of air. Dust collected during this operation contained 100 ppb aflatoxin and  $2.1 \times 10^7$  A. flavus propagule/g. After storage, the clean corn containing 360 ppb aflatoxin was transferred again and 16.8  $\mu\text{g}$  of aflatoxin and  $4.0 \times 10^5$  A. flavus propagules were recovered/ $\text{m}^3$  of air at the transfer site. To relate these findings to human and animal health is difficult in such a limited study. Nevertheless, the normal adult inspires approximately 6 l of air/min. Consequently, a minimum 2-3 hr constant exposure would be required for an adult to inspire the contents of  $1 \text{ m}^3$  of air. During preparation in the laboratory of aflatoxin-contaminated samples for analytical analysis, aflatoxin was not detected in numerous air samples. The safety precautions (hoods, incinerator, etc.) taken in the laboratory, apparently eliminated any airborne toxin hazard.

Corn-soy-milk was inoculated ( $10^3$  cells/g) with Salmonella senftenberg and S. anatum. S. senftenberg was more thermal tolerant than S. anatum. Nevertheless, at 43, 49, 54, and 60°C, S. senftenberg was inactivated after 13, 10, 8, and 6 days, respectively. (See also ED, A.3.

#### Publications:

BOTHAST, R. J., L. T. BLACK, L. L. WILSON, AND E. E. HATFIELD.  
Methylene-bis-propionate (MBP) Preservation of High-moisture Corn.  
J. Anim. Sci. 46 (1978):484-489.

HESELTIME, C. W., R. F. ROGERS, AND R. J. BOTHAST. A microbiological study of exported soybeans. Cereal Chem. 55 (1978):332-340.

BOTHAST, R. J., L. R. BEUCHAT, B. S. EMSWILER, M. G. JOHNSON, AND M. D. PIERSON. Incidence of Airborn Aspergillus flavus Spores in Cornfields of Five States. J. Appl. Environ. Microbiol. 35 (1978):627-628.

BOTHAST, R. J. Fungal Deterioration and Related Phenomena in Cereals, Legumes, and Oilseeds. In Post Harvest Biology and Biotechnology, Edited by H. O. Hultin, Food and Nutrition Press, Inc., Westport, Connecticut, 1978.

BOTHAST, R. J. Treating Aflatoxin-contaminated Grain. In Proceedings Purdue Feed Industry Conference, February 21-22, 1978.

RAMSTACK, J. M., E. B. LANCASTER, AND R. J. BOTHAST. Gas Chromatographic Headspace Analysis of Solid Substrate Fermentations. Process. Biochem., accepted April 25, 1978.

McGHEE, J. E., E. B. BAGLEY, AND R. J. BOTHAST. Treating Corn with Aqua Ammonia: Effect on Meal Constituents. Cereal Chem., accepted June 15, 1978.

BOTHAST, R. J. AND E. E. HATFIELD. Preservation of High-moisture Corn. In Proceedings 1977 Corn Quality Conference, Champaign, Illinois. February 1978. (Abstract)

BOTHAST, R. J., R. F. ROGERS, AND C. W. HESSELTINE. Fungal Deterioration of Bags During International Transport of Corn-soya-milk: A Test Shipment. J. Food Sci., accepted July 17, 1978.

KARKI, T. B., R. J. BOTHAST, AND R. D. STUBBLEFIELD. Microbiological and Aflatoxin Analysis of Cereal Grains from the Tarai Plain of Southern Nepal. Cereal Chem., accepted September 29, 1978.

PEPLINSKI, A. J., O. L. BREKKE, R. J. BOTHAST, AND L. T. BLACK. High-moisture Corn--An Extended Preservation Trial with Ammonia. Trans. ASAE, 21 (1978):773-776,781.

SILMAN, R. W. Production of  $\alpha$ -Galactosidase and Invertase by Aspergillus awamori NRRL 4869 on Solid Substrate Medium in Rotating Vessels with Controlled Aeration. Biotechnol. Bioeng., to be submitted.

#### Other Reports:

BOTHAST, R. J. Extending the Allowable Drying Time for Ambient Conditioned High-moisture Corn. Presented at the Sixth International Cereal and Bread Congress, Winnipeg, Canada, September 16-22, 1978.

BOTHAST, R. J. Microbiological Changes During International Transport of Soybeans and CSM. Presented at Food Protein Council Scientific Committee Meeting, Peoria, Illinois, September 27, 1978.



C. NATURAL TOXICANTS AND MICROBIAL  
TOXINS

1. Studies on Mycotoxins in Cereal Grains and Their Control (R. A. Anderson)

See Fermentation Laboratory, G.1.

## FERMENTATION LABORATORY

C. W. Hesseltine, Chief

Research Leaders: R. W. Detroy, T. G. Pridham, O. L. Shotwell,  
and M. E. Slodki

### A. PHYSIOLOGICAL AND BIOCHEMICAL TECHNOLOGY TO IMPROVE CROP PRODUCTION

#### 1. Relationships of Nitrogen Fixation in Microorganisms and Plants to Reduce Energy Requirements of Crops (J. W. Newton)

- a. Specific Objective: Compare heterotrophic and autotrophic nitrogen fixation using free-living blue-green algae and those strains isolated from Azolla and to study enzymes of nitrogen metabolism in both algal types.

Progress: Blue-green algae isolated from Azolla were found to grow heterotrophically in the dark with fructose as an energy source. This heterotrophic growth is not a general property of blue-green algae and indicates that those isolated from symbiotic nitrogen-fixing systems may be more heterotrophic than free-living strains. Furthermore, the heterotrophically grown strains had two- to threefold higher specific activity of nitrogenase than when they were grown autotrophically in the light. This difference was not displayed by a strain of Anabaena flos-aquae which can also grow heterotrophically. D-Fructose was the principal free sugar found in leaf extracts. Neither the free-living alga nor the isolated symbionts excreted detectable amounts of ammonia. There were no differences in glutamine synthetase activity and in ammonia excretion in the presence of the inhibitor methionine sulfoximine.

- b. Specific Objective: Develop a genetic transfer system for introduction of nitrogen fixation into nontumorigenic Agrobacterium strains.

Progress: To facilitate genetic conjugation experiments, two procedures were worked out for preparation of spheroplasts from rhizobia and agrobacteria: Polymyxin B/lysozyme and D-methionine/lysozyme. At this time, the objective was altered to transfer of derepressed, free-living nitrogenase activity between rhizobia. Root nodulation tests were done with selected rhizobia that give such nitrogenase activity in order to determine the range of host specificity and utility for future experiments.

Quantitative gas diffusion measurements ( $O_2$ ,  $H_2$ , acetylene,  $N_2$ ) enabled precise definition of the microaerobic condition necessary for expression of nitrogenase by free-living rhizobia. Nitrogenase activity was measured by both reduction of acetylene and incorporation of  $^{15}N_2$  into cells. The lower efficiency of the free-living rhizobia



(acetylene: $^{15}\text{N}_2 \sim 12$ ) compared to normally free-living nitrogen fixers (acetylene: $^{15}\text{N}_2 \sim 3$ ) can, in part, be accounted for by loss of metabolic reducing power through evolution of molecular hydrogen. Loss of nitrogen through excretion as soluble, nonprotein N could not be detected.

Rhizobium sp. 32H1 (NRRL B-4328) is commonly used to study free-living nitrogenase activity, but this response has been erratic in our test system. It was found that there is a requirement for cobalt ion, glycerol, and gluconate in a casamino minimal medium. Ascorbate replaced cobalt ion and glycerol. The metal ion may be involved in polyol metabolism; it is theorized that these substrates in some way affect the cell membrane. Other rhizobia simply require mannitol and gluconate while cobalt ion inhibits.

Rhizobium japonicum strain 3Ilb 123, indigenous to midwest soils, is considered to be an inefficient nitrogen fixer. It did not reduce acetylene under our conditions. In contrast, highly efficient strains of the species from SEA-AR's Beltsville Agricultural Research Center displayed moderate reduction.

- c. Specific Objective: Further study any proteins which appear to be unique plant components characteristic of nitrogen-fixing symbioses.

Progress: Two apparatuses were designed and built for vertical polyacrylamide slab gel electrophoresis. Experiments are currently in progress; resolution and definition are excellent.

- d. Specific Objective: Attempt to isolate tissue culture lines of the water fern Azolla free of microbial infestation.

Progress: Successful isolation of tissue cells free of contamination was achieved from leaves washed with sodium hypochlorite in nonionic detergent. The callus, however, dies off. Perhaps some unknown nutritional requirement has not been met or some trace constituent is at fault.

- e. Specific Objective: Determine the effects of tissue culture medium on the growth of free-living Anabaena azollae.

Progress: There is no effect of tissue culture medium on the Anabaena azollae. An attempt was made to obtain strains resistant to the antibiotics penicillin and streptomycin, but stable variants could not be isolated. In the course of this work, examination of A. flos-aquae and A. azollae with nuclear strains revealed heretofore undescribed cytological forms: the heterocysts are not all identical in nuclear staining and internal structure. Unique forms occur early in the growth cycle and some of these might be invasive.

- f. Specific Objective: Study electron transport in nitrogen-fixing microorganisms as it relates to both nitrogenase and evolution of hydrogen.

Progress: Metronidazole (2-methyl-5-nitroimidazole-1-ethanol) is a synthetic inhibitor of anaerobic bacteria. Its possible role as a specific inhibitor of low-potential electron transport to nitrogenase was examined for both anaerobic and aerobic nitrogen-fixing organisms: In Clostridia, there appears to be a shared electron transport pathway for both H<sub>2</sub> production and nitrogen fixation. Separate pathways appear to be present in Klebsiella and in blue-green algae. Killing of the aerobe Azotobacter was observed only in the absence of a combined nitrogen source; i.e., under nitrogen-fixing conditions. These results indicate that metronidazole can serve as a new tool for study of electron transport to nitrogenase.

#### Publications:

KANESHIRO, T., C. D. CROWELL, AND R. F. HANRAHAN, JR. Acetylene Reduction Activity in Free-Living Cultures of Rhizobia. Intern. J. Syst. Bacteriol. 28 (1978):27-31.

KANESHIRO, T., J. W. NEWTON, E. SELKE, AND M. E. SLODKI. Comparison of <sup>15</sup>N<sub>2</sub> Fixation and Acetylene Reduction in Free-Living Rhizobia. Submitted to Appl. Environ. Microbiol.

NEWTON, J. W. The Relationship Between Photosynthesis and Nitrogen Fixation Revisited. Accepted for publication by Academic Press in "From Cyclotrons to Cytochromes: A Symposium Honoring Martin D. Kamen."

NEWTON, J. W., AND A. I. HERMAN. Isolation of Cyanobacteria from the Aquatic Fern, Azolla. Arch. Microbiol., 1979. Accepted for publication.

NEWTON, J. W., D. D. TYLER, AND M. E. SLODKI. Effect of UV-B (280-320 nm) radiation on blue-green algae (cyanobacteria), possible biological indicators of stratospheric ozone depletion. Submitted to Appl. Environ. Microbiol.

#### Other Reports:

KANESHIRO, T., E. SELKE, AND M. SLODKI. Nitrogen Fixation of Free-Living Rhizobia Detected by <sup>15</sup>N Enrichment. Abstr. Joint Central-Great Lakes Regional Meeting of the American Chemical Society, May 24-26, 1978, 40.

NAGATANI, H. H. Effect of Flagyl (Metronidazole) upon Nitrogen Fixation in Free-Living Bacteria. Abstr. Steenbock-Kettering International Symposium on Nitrogen Fixation, June 12-16, 1978, 10.

NEWTON, J. W. Physiology of Blue-Green Algae (Cyanobacteria) Isolated from Azolla. Abstr. Steenbock-Kettering International Symposium on Nitrogen Fixation, June 12-16, 1978, 19.

2. Polysaccharides in Specific Associations of Nitrogen-Fixing Microbes with Plants (M. E. Slodki)

- a. Specific Objective: Elucidate structure of Rhizobium sp. B-4384 capsular polysaccharide.

Progress: Per-0-methylation of the polysaccharide (PS B-4384) converted both L-rhamnose and unknown methylpentose components to 2,3,4-tri-0-methyl rhamnose. Resistance of the unknown sugar to periodate oxidation and mass spectrometry of its per-0-acetyl aldononitrile and of mixed per-deuteriomethylated alditols of it and L-rhamnose established 0-methyl substitution of the C-3 hydroxyl. The 3-0-methyl methylpentose was isolated by chromatography on a column of powdered cellulose and crystallized. Optical rotation and melting point confirm 3-0-methyl-L-rhamnose, hitherto unknown as a constituent of rhizobial polysaccharides. In similar fashion, the uronic acid component was isolated and identified as D-galacturonic acid. D-Galactose oxidase was used to quantitate and identify the D-galactose in the intact PS after the carboxyl was reduced. The D-glucose component in hydrolyzates was similarly confirmed by use of D-glucose oxidase.

Per-0-methylation, in addition to demonstrating that the methylpentoses occur as nonreducing end groups, revealed the presence of (1 → 3)-linked glucosyl and mannosyl residues as chain-extending units; 1,3,4- and 1,3,6-tri-0-substituted hexopyranosyl residues are points of branching. Subsequent to this analysis, the acidic PS was separated as a quaternary ammonium salt from two minor neutral PS (glucan and mannan). Studies on cell-bound PS B-4384 showed that it is not 0-acylated.

- b. Specific Objective: Isolate and characterize lipopolysaccharides of R. japonicum B-4421 and B-4422.

Progress: The lipopolysaccharides (LPS) were isolated and fractionated according to J. Maier and W. J. Brill [J. Bacteriol. 133 (1978):1295]. Analysis of sugar composition made it clear that all fractions obtained by these investigators were contaminated by exocellular capsular polysaccharide (PS). Because recent reports from other laboratories now strongly implicate PS and not LPS in the recognition process, the work was redirected toward PS. Fractionation of PS from both strains gave acidic PS containing L-rhamnose and 4-0-methyl-D-glucuronic acid as sole sugar components. Meanwhile, W. F. Dudman [Carbohydr. Res., 66 (1978):9] had published the structure of PS B-4421 (wild type). Optical rotations of PS B-4421 and PS B-4422 are similar to the published value. As with PS B-4384, the minor, neutral fractions were glucan and mannan. Titration of PS B-4421 and the mutant PS B-4422 gave equivalent weights of 409 and 334, respectively. Carboxyl reduction and hydrolysis gave respective molar ratios of L-rhamnose:4-0-methyl-D-glucose of 3:1 and 2:1. The former value agrees with Dudman's. Both PS lack 0-acyl groups.



- c. Specific Objective: Obtain from lipopolysaccharides oligosaccharide fragments that could be useful in determining specific lectin-binding sites.

Progress: In line with the change in emphasis toward capsular polysaccharide, conditions have been worked out for maximum yield of oligosaccharides by mild acidic hydrolysis.

- d. Specific Objective: Initiate work on structure of root cap slime.

Progress: With hybrids of field and sweet corn, various factors were examined for production of root cap slime on sucrose and fructose: incubation temperature (25° and 28°), agitation, volume/root ratio and bactericidal/fungicidal agents. Slimes were isolated by precipitation with ethanol. Field corn, especially with sucrose as substrate, gives much higher yield of slime. In contrast to the results of others, uronic acids were present only in trace amounts. Major components were fructose, arabinose, xylose, mannose, glucose, and galactose; an unidentified major component was present in slime from field corn root tips incubated in fucose at 28°. As in the literature, at 28° there is a dramatic increase in either glucose or fucose content when incubated in sucrose and fucose, respectively. These changes were not observed at 25°. Incubation in fucose at 25°, however, led to an increase in xylose.

Slimes were fractionated by precipitation of acidic polysaccharides as quaternary ammonium salts. The neutral components were further purified by fractional precipitation with ethanol. In this manner, a hitherto undescribed fucan was isolated from the slime produced by field corn root tips incubated in the sucrose medium.

- e. Specific Objective: Cooperate with Charles F. Kettering Research Laboratory in structure determination of Rhizobium japonicum 3Ilb 138 exopolysaccharide (PS 138).

Progress: The methylated galactose component of PS 138 was identified as 4-0-methyl-D-galactose by mass spectrometry of the per-0-acetyl aldonoitrile and identical g.l.c. retention time of this derivative in a mixture derived from partial methylation of  $\alpha$ -methyl-D-galactopyranoside. Permethylation of unreduced acidic PS 138 was apparently successful as judged by the correspondence between 2,3,4,6-tri-0-methyl ether (apparently from the 4-0-methyl galactosyl residue) and 2,4-di-0-methyl-D-glucose.

Approximate molar ratios of 2,3,4,6-tri-0-methyl galactose:2,4,6-tri-0-methyl glucose:2,4,6-tri-0-methyl mannose:2,4,6-tri-0-methyl galactose:2,4-di-0-methyl glucose are, respectively, 1:1:1:0.3:1. It appears that PS 138 may have a backbone structure similar to that of PS B-4384. Both biopolymers contain D-galacturonic acid. It should be noted that R. japonicum 138 is a competitive, but less efficient, nitrogen fixer found in midwest soils.

## Publications:

CADMUS, M. C., C. A. KNUTSON, A. A. LAGODA, J. E. PITTSLEY, AND K. A. BURTON. Synthetic Media for Production of Quality Xanthan Gum in 20-Liter Fermentors. *Biotechnol. Bioeng.* 20 (1978):1003-1014.

SLODKI, M. E., AND M. C. CADMUS. Production of Microbial Polysaccharides. *Adv. Appl. Microbiol.* 23 (1978):19-54.

SANDFORD, P. A. Exocellular Microbial Polysaccharides. *Adv. Carbohydr. Chem. Biochem.* 36 (1979), in press.

## B. BIOLOGICAL AGENTS FOR PEST CONTROL

### 1. Biochemistry of Toxin Production in *Bacillus thuringiensis* (D. E. Johnson)

- a. Specific Objective: Determine the size and number of toxic subunit moieties of the delta-endotoxin from *Bacillus thuringiensis*, using the insect cell culture bioassay system. Also investigate *B. thuringiensis* spores for similar toxic proteins that may be located in the spore coat.

Progress: Six asporogenous but crystal-forming mutants of *B. thuringiensis* subsp. *kurstaki* were isolated after mutagenesis with nitrosoguanidine. These isolates provide an alternative for the collection of parasporal crystals free of any spore contamination. Any subsequent biochemical work with dissolved endotoxin protein can, therefore, be considered free of spore protein contaminants. The entomocidal activity of parasporal crystals prepared from each of these isolates was measured in vitro using cultured insect cells of the spruce budworm, *Choristoneura fumiferana*. The crystals, first separated from other cellular debris by buoyant density centrifugation, were then solubilized in thioglycollate buffer followed by enzymatic digestion with  $\alpha$ -chymotrypsinogen in order to artificially duplicate gut proteolysis. The toxic activity of the resultant digests, as measured with an insect cell-ATP bioassay, ranged from an LD<sub>50</sub> of 4.5 to 10.5  $\mu$ g protein/ $2 \times 10^5$  cells. Since the parent strain (wild type) exhibited an LD<sub>50</sub> of 13.4  $\mu$ g protein/ $2 \times 10^5$  cells after proteolysis, the mutant strains produce a parasporal crystal very similar, if not identical, to the parent. Therefore, these mutants can be used as a source of parasporal protein uncontaminated by spores or spore protein for biochemical work and composition studies.

- b. Specific Objective: Accomplish microencapsulation of *B. thuringiensis* spores and crystals or dissolved and digested crystal product for use against resistant lepidoptera pests and other insects not susceptible to the intact crystal.

Progress: An alternate process for applying wood rosin as an encapsulant for *B. thuringiensis* has been developed to lower the ratio of rosin to *B. thuringiensis* and to eliminate the undesirable high-



alkalinity rosin solubility step involved in the previous encapsulation process. Rosin is now dissolved in ethyl alcohol and sprayed over the dry insecticide. Such preparations are currently being field-tested against Japanese beetle larvae by SEA-AR scientists at Wooster, Ohio.

The foam flotation technique for the separation of bodies with similar densities has been considerably improved by the addition of gelatin as a foaming agent. The improvement in the technique has been used to separate spores of B. thuringiensis from parasporal crystals.

- c. Specific Objective: A new variant strain of B. popilliae having unusual morphological characteristics has been isolated which also has a significantly reduced infectivity rate toward Japanese beetle larvae. Examine ultramicroscopically for visible changes in the parasporal structure which might lead to reduced infectivity.

Progress: A considerable amount of ultramicroscopy has been completed with both B. popilliae and B. thuringiensis. Transmission electron microscopy (TEM) of the paraspore from B. popilliae using carbon replicas revealed the same crystalline array on its surface as is found with B. thuringiensis parasporal crystals, thus suggesting a similar structural relationship between the two paraspores. Additional TEM of the parasporal crystal from B. thuringiensis subsp. kurstaki by both thin section and carbon replicas has revealed the presence of a unique refractile inclusion body which exists as an integral part of the parasporal crystal. The inclusion body, which is visible by normal phase-light optics, leaves a void or depression in the crystal upon its removal. Two distinct shapes are oftentimes seen (ovoid and a square, flat, disc-like shape), and neither possess a surface crystalline structure like that of the larger paraspore.

- d. Specific Objective: To continue screening for fungi and bacteria that may be pathogenic or toxic to scarab beetle larvae.

Progress: The screen has been completed. The following microorganisms were tested for their infectivity or toxicity to Popillia japonica larvae: B. megaterium, B. apiarius, B. alvei, B. cereus, B. subtilis, B. thuringiensis, Metarrhizium anisoplaeae, M. brunneum, and Beauveria bassiana. Spores of each of the microorganisms were fed at concentrations of  $1 \times 10^9$ ,  $1 \times 10^7$ ,  $1 \times 10^5$ , and  $1 \times 10^3$  per gram of soil. In general, the sporeforming bacteria killed an average of 30% of the larvae at the two highest spore concentrations. Of the larvae that were killed, only about 3% contracted a bacterial infection, which suggests that larval death was due to bacterial toxin in the majority of cases. One strain of B. thuringiensis (NRRL B-2245) killed 50% of the larvae, but this effect could not be repeated in two other trials. Both fungi (Metarrhizium and Beauveria) killed about 20% of the larvae. The fungi exhibited the classical infection pattern.



### Publications:

JOHNSON, D. E. Inhibition of RNA Polymerase from Bacillus thuringiensis and Escherichia coli by  $\beta$ -Exotoxin. Can. J. Microbiol. 24 (1978):537-543.

ST. JULIAN, G., L. A. BULLA, JR., AND R. W. DETROY. Stored Bacillus popilliae Spores and Their Infectivity Against Popillia japonica Larvae. J. Invertebr. Pathol. 32 (1978):258-263.

SHARPE, E. S. AND L. A. BULLA, JR. Characteristics of the Constituent Substrains of Bacillus popilliae Growing in Batch and Continuous Cultures. Appl. Environ. Microbiol. 35 (1978):601-609.

### Other Reports:

ST. JULIAN, G. Nomuraea rileyi Conidia Production and Their Infectivity Against Popillia japonica Larvae. Presented at Entomological Society of America Meeting, Houston, Texas, November 26-30, 1978.

ST. JULIAN, G. Problems Involving Mass Production of Fungal Insecticides. Presented at the SEA-AR Annual Insect Pathology Workshop, Iowa State University, Ames, Iowa, October 31-November 3, 1978.

ST. JULIAN, G. SEA-AR Research Opportunities. Presented at the National Technical Association and Career Conference, Bradley University, Peoria, Illinois, March 20, 1978.

ST. JULIAN, G. Regulation of Insect Populations by Microorganisms. Presented at Prairie View Agriculture and Mechanical University, Prairie View, Texas, November 28, 1978.

ST. JULIAN, G. NRRC Organization and Functions. Presented at Texas Southern University, Houston, Texas, November 30, 1978.

SHARPE, E. S. Use of Encapsulated Bacillus thuringiensis Against Japanese Beetle Larvae and Bacteriological Changes Accompanying Variation in Host Preference of Milky Disease in Connecticut and Ohio. Presented at the SEA-AR Insect Pathology Workshop, Iowa State University, Ames, Iowa, October 31-November 3, 1978.

### C. TECHNOLOGIES FOR FOOD AND FEED USES OF FIELD CROPS

#### 1. Germ Plasm Bank of Microorganisms for Research in Cereal Microbiology (T. G. Pridham)

- a. Specific Objective: To continue operation of the Agricultural Research Culture Collection.

Progress: Mycology staff members of the Agricultural Research Culture Collection continued acquiring, maintaining, and distributing cultures

and information; their systematic studies; and their supportive and original research. As of January 1, 1979, the Collection maintained 58,479 strains of molds, yeasts, bacteria, actinomycetes, and algae. During 1978, the Collection distributed 3,201 strains of which 1,824 were sent to investigators in the United States and 1,377 were sent abroad. Of 136 strains deposited in the patent collection, 78 were from foreign sources; 200 patent strains were distributed to United States researchers and 236 to foreign. Three hundred nine strains of molds and yeasts were identified to species for interested parties.

Curators of the Collection continued to contribute publications on systematics of yeasts and molds by collating and up-dating information on occurrence, characterization, identification, and classification.

DNA studies of strains of the genus *Issatchenkia* showed that, despite a low order (20-25%) of DNA reassociation suggesting nonrelationship, mating studies were successful. This finding places some question on conclusions that low orders of DNA reassociation definitely indicate nonidentity. Larvae of the sciarid fly *Lycoriella mali* do not feed on ascocarps of *Chaetomium bostrycodes* on rabbit dung but do on other coprophilous molds. Studies of the morphology of *C. bostrycodes* suggest that the perithecial hairs may have evolved as a mechanical deterrent against predation allowing sporulation on rich, short lived nutrients such as dung.

Paired cultivation of various molds from corn with a strain of *Aspergillus flavus* not exhibiting the characteristic blue-green-yellow fluorescence (BGYF) alone led to development of the fluorescence. It was concluded that any field or storage-related microenvironment suitable for fungal biosynthesis of kojic acid and peroxidases in cereal grains can result in formation of BGYF. The findings provide indirect evidence that microfungal colonization patterns enable *A. flavus* to colonize cereal grains in the field and to produce significant levels of aflatoxin and kojic acid.

Work was continued and new phases begun on studies of various methods for preservation of microbial germplasm, especially that which defies lyophilization. Storage of agar blocks permeated with mold hyphae in sterilized distilled water shows promise. Strains of *Hymenomycetes* and *Entomophthorales* survive such storage at 25 C for 20 months. Use of liquid nitrogen, silica gel, and mineral oil as aids to preservation has begun. Results are dependent on passage of time and appropriate viability checks. Basic phases of the lyophilization process have been identified and a suitable base line process developed to help identify parameters vital to successful lyophilization.

Expertise of the curator of *Phycomycetes* in identifying these molds when associated with phycomycoses in humans and animals continues to provide significant aid to members of the scientific and medical community and to contribute to the overall systematics research effort. The occurrence of these molds on cereal grains and oilseeds

and their role as etiological agents emphasizes the value of systematics research in contributing to solution of both medical and nonmedical problems caused by these molds.

Publications:

ELLIS, J. J., D. I. FENNELL, AND C. W. HESSELTINE. Collections of Fungi in the United States. USDA, ARM Publication, in press.

KURTZMAN, C. P. Hemiascomycetes. In Encyclopedia on Taxonomy and Classification of Living Organisms. McGraw-Hill Publishing Company, in press.

NAKAMURA, L. K. AND C. W. HESSELTINE. Solid and Solidified Growth Media for Microorganisms. In CRC Handbook Series in Nutrition and Food, Edited by M. Recheigl Jr., CRC Press, Inc., West Palm Beach, Florida, in press.

PRIDHAM, T. G. Micro-organism Culture Collections: Acronyms and Abbreviations. Additions through December 1978. ARS-NC-17, 12 pp.

PRIDHAM, T. G. References to Works on Formation of Pellets, Nodules, or Fungus Balls by Molds in Submerged Culture. Multilithed for distribution (1978) 3 pp.

WICKLOW, D. T. Hair Ornamentation and Predator Defense in Chaetomium. Trans. Brit. Mycol. Soc., in press.

WICKLOW, D. T. Interference Competition and the Organization of Fungal Communities: Its Organization and Role in the Ecosystem, in press.

WICKLOW, D. T. Identification of Aspergillus and Penicillium Species: Workshop. Phytopathol. News 12 (1978):115.

WICKLOW, D. T. AND G. C. CARROLL (Eds.). The Fungal Community: Its Organization and Role in the Ecosystem, in press.

WICKLOW, D. T. AND J. C. ZAK. Ascospore Germination of Carbonicolous Ascomycetes in Fungistatic Soils: An Ecological Interpretation. Mycologia, in press.

2. Characteristics and Classification of Rhizopus-like Fungi from Corn and Other Cereal Grains (C. W. Hesseltine)

- a. Specific Objective: Collect and study isolates of Rhizopus and related molds from a wide variety of sources, especially cereal grains.

Progress: During the reporting period, 167 new isolates were accessioned belonging to Rhizopus and 13 related genera. Fourteen of the isolates, most Rhizopus arrhizus, came from sunflower seed or rotting



sunflower heads. Other substrate sources were barley, ground linseed, millet, milo, prairie dock seed, wheat, arrhizus, Ragi, soil, herbivore and rodent dung, ant fungus garden, patients, and larval cadavers of the alfalfa weevil. These isolates will increase our reservoir for further detailed studies related to improved quality of food and feed as well as certain aspects of animal and human health.

- b. Specific Objective: Distinguish growth parameters of certain species of Rhizomucor and Rhizopus known to produce enzymes such as microbial rennet or amylase and also known as potential opportunistic animal pathogens.

Progress: A total of 23 strains of Rhizopus arrhizus, Rhizopus oryzae, Rhizomucor pusillus, and Rhizomucor miehei were grown in liquid media to test their ability to utilize each of 14 carbon sources at a 10 g C/L level. The 10 strains of Rhizopus, including both species, showed significantly less dry weight when grown on sucrose, raffinose, L(-) sorbose, lactose, soluble starch or glycogen than when grown on any of the other carbon sources used. Strains of Rhizomucor pusillus and Rhizomucor miehei exhibited significantly different growth patterns between the two species. A homothallic strain of Rhizomucor pusillus (in which most strains are heterothallic) exhibited a growth pattern similar to Rhizomucor pusillus rather than to Rhizomucor miehei (in which all strains are homothallic). These data add physiological evidence to the limited morphological evidence to support the distinction of the two species and to correctly place the one homothallic strain.

The 23 strains were also checked for growth in media at 4 levels of a C:N ratio from 3:1 to 20:1. There was no apparent increase in growth above a 10:1 level when 2 g C/L asparagine was used and the concentration of glucose varied. When these strains were grown on various levels of salts in the synthetic medium, the salts could be reduced to at least a 1:16 level without any change in amount of growth on a dry weight basis.

#### Publications:

O'DONNELL, K. L., S. L. FLEGLER, J. J. ELLIS, AND C. W. HESSELTINE. The Zygorhynchus Zygosporangium and Zygospore. Can. J. Bot. 56 (1978):1061-1073.

#### Other Reports:

HESSELTINE, C. W. Thirty Years of Adventure with the Fungi. Pasteur Award Address, October 14, 1978, Chicago, Illinois.

### 3. Conversion of Cellulosic Materials to Ruminant Feed by Solid Substrate Fermentation (R. W. Detroy)

- a. Specific Objective: Mutant and variant fungi will be used to selectively degrade the lignin in wheat straw thereby releasing the cellulose from further digestion by ruminant animals.

Progress: In research with a lignin-cellulose-degrading white-rot fungus, Phanerochaete chrysosporium, many mutant isolates were screened for their ability to degrade only lignin, not cellulose. Cellulase (-) mutants as presumptives are being tested for their ability to degrade the lignin component only of wheat straw lignocellulosics.

- b. Specific Objective: Evaluate the fermentation capability of white-rot fungi to degrade lignocellulosic, agricultural residues as feedstuff for ruminants.

Progress: Experimentation with the oyster mushroom indicated that this organism was capable of degrading wheat straw residues substantially, as evidenced by 45% and 55% decreases in lignin and cellulose, respectively, after 36 days. Solid substrate fermentations were conducted with native wheat straw with various N amendments. Using an in vitro digestibility assay, the enhanced nutritional quality of the fermented straws were evaluated. Digestibility increased three-fold after a 90-day fermentation period. Optimum incubation conditions were determined for this type of fermentation so as to be useful on farm situations.

Publications:

DETROY, R. W. AND C. W. HESSELTINE. Availability and Utilization of Agricultural and Agro-Industrial Wastes. Process Biochem. 13 (1978): 2-10.

LINDENFELSER, L., R. DETROY, J. RAMSTACK, AND K. WORDEN. Biological Modification of the Lignin and Cellulose Components of Wheat Straw by Pleurotus Ostreatus. Dev. Ind. Microbiol., Vol. 19, March 1979.

4. Rapid Characterization of Yeasts Through Genetic and DNA/DNA Hybridization and Computer Analysis (C. P. Kurtzman)

- a. Specific Objective: Clarify the taxonomy of the genus Sterigmatomyces.

Progress: Since the last reporting period, additional DNA reassociation experiments were carried out, but the whole study was not completed.

- b. Specific Objective: Determine whether previously uncharacterized species of Pichia and Hansenula are homothallic or heterothallic.

Progress: Of the approximately 90 known species of Pichia and Hansenula, the mating system of 60 has been determined. Species found to be heterothallic were tested for interspecific mating response in order to clarify species boundaries.

- c. Specific Objective: Contribute six chapters to the taxonomic reference book, The Yeasts. Chapters will be on the genera Pichia, Hansenula, Lipomyces, Citeromyces, Pachysolen, and Issatchenkia.



Progress: Manuscripts on the genera Lipomyces, Citeromyces, Pachysolen, and Issatchenkia were completed, and the chapters on Pichia and Hansenula are undergoing final revision.

#### Publications:

KURTZMAN, C. P. Salad Dressings. In Compendium of Methods for the Microbiological Examination of Foods, Edited by M. L. Speck, Chapter 48, American Public Health Association, Washington, D.C. Revised edition, in press.

KURTZMAN, C. P., C. J. JOHNSON, AND M. J. SMILEY. Determination of Conspecificity of Candida utilis and Hansenula jadinii through DNA Reassociation. Mycologia, in press.

KURTZMAN, C. P. AND D. T. WICKLOW. Mycology. In Highlights in Microbiology, 1977-1978, Edited by D. Vesley and R. J. Moon. American Society for Microbiology, Washington, D.C., in press.

KURTZMAN, C. P. Hemiascomycetes. In Taxonomy and Classification of Living Organisms, Edited by S. P. Parker, McGraw-Hill Book Co., New York, accepted.

KURTZMAN, C. P. Citeromyces Santa Maria. In The Yeasts, A Taxonomic Study, 3rd Ed., Edited by N. J. W. Kreger-van Rij. North-Holland Publishing Co., Amsterdam, accepted.

KURTZMAN, C. P. Pachysolen Boidin et Adzet. In The Yeasts, A Taxonomic Study, 3rd Ed., Edited by N. J. W. Kreger-van Rij. North-Holland Publishing Co., Amsterdam, accepted.

KURTZMAN, C. P. AND M. J. SMILEY. Taxonomy of Pichia carsonii and its Synonyms Pichia vini and P. vini var. melibiosi: Comparison by DNA Reassociation. Mycologia, in press.

PHAFF, H. J. AND C. P. KURTZMAN. Lipomyces Lodder et Kreger-van Rij. In The Yeasts, A Taxonomic Study, 3rd ed., Edited by N. J. W. Kreger-van Rij. North-Holland Publishing Co., Amsterdam, accepted.

#### Other Reports:

KURTZMAN, C. P. Culture Deposits for Patents. Presented at the American Society for Microbiology meeting, Las Vegas, Nevada, May 13-19, 1978.

KURTZMAN, C. P. The Genus Issatchenkia: Taxonomy and Imperfect States. Presented at the U.S. Federation for Culture Collections meeting, American Society for Microbiology meeting, Las Vegas, Nevada, May 13-19, 1978.

KURTZMAN, C. P. Long-term Viability of Fungi Under Different Methods of Maintenance. Presented at XII International Congress of Microbiology, Munich, Germany, September 3-8, 1978.



PRIDHAM, T. G. Maintenance of Non-Sporulating Molds. Poster presentation, XII International Congress of Microbiology, Munich, Germany, September 3-8, 1978.

5. Germ Plasm Bank of Microorganisms for Research on Plant Residue Utilization (T. G. Pridham)

- a. Specific Objective: To continue operation of the Agricultural Research Culture Collection and determine the taxonomic status of 80 lactobacilli isolated from swine waste-corn fermentations.

Progress: Bacteriology staff members of the Agricultural Research Culture Collection continued acquiring, maintaining, and distributing cultures and information; their systematic studies; and their supportive and original research. As of January 1, 1979, the Collection maintained 58,479 strains of molds, yeasts, bacteria, actinomycetes, and algae. During 1978, the Collection distributed 3,201 strains of which 1,824 were sent to investigators in the United States and 1,377 were sent abroad. Of 136 strains deposited in the patent collection, 78 were from foreign sources; 200 patent strains were distributed to United States researchers and 236 to foreign. Fifty-three strains of bacteria and actinomycetes were identified to species for interested parties.

Further taxonomic study of the 80 homofermentative lactobacilli isolated from swine waste-corn fermentations resulted in proposal of the new species, Lactobacillus amylophilus for 24 strains. Lactobacillus amylophilus is a species which can hydrolyze starch, an unusual characteristic for lactobacilli. The remaining 56 strains were identified as other more common species of lactobacilli.

A transmission electron microscope study of 5,900 strains of aerobic Actinomycetales was completed which allows identification to species for most of the strains in the Collection.

Further progress was made in developing a rapid counting method for eufilamentous Actinomycetales. Three media have been selected to serve as master controls in continuation of this work.

Several lots of crambe meal were found satisfactory as replacement for soybean meal in conventional fermentation mashes. A variety of microorganisms were found able to grow in the mashes and secondary metabolite production by two strains was verified.

Progress was made on preparation of the Approved List of names of streptomycetes and streptovercillia beyond the first draft stage.

The list of acronyms of culture collections throughout the world was updated to include new acronyms found in use during 1978.

Progress was made on systematic studies of streptomycetes and streptovercillia through continuing accumulation of data on whole-cell

hydrolyzate analyses, morphologies, electron microscopy, and antibiotic activity patterns.

Contributions were made, as faculty members, to a national Workshop on Actinomycete Taxonomy sponsored by the Society for Industrial Microbiology.

Negotiations were begun to conclude agreements with the European Patent Office, the Swedish Patent Office, and the World Intellectual Property Organization which will result in the Agricultural Research Culture Collection being designated as an official International Depositary Authority in connection with patent strain depositions.

#### Publications:

NAKAMURA, L. K. Deposition of Microorganisms: Agricultural Research Service. Dev. Ind. Microbiol. 19 (1978):161-167.

NAKAMURA, L. K. AND C. D. CROWELL. Lactobacillus amylophilus, A New Starch-Hydrolyzing Species From Swine Waste-Corn Fermentation. Dev. Ind. Microbiol. (accepted).

PRIDHAM, T. G. AND A. J. LYONS. Methodologies for Actinomycetales with Special Reference to Streptomyces and Streptoverticillia. Soc. Ind. Microbiol. Special Publ. (accepted).

#### Other Reports:

LYONS, A. J. Methodologies for Streptomyces and Streptoverticillia: Inoculum, Whole-Cell Hydrolyzate Analyses, Inoculation; Electron Microscopy, Carbon Utilization, Spectrum Dishes, Cross-Antagonism Dishes. Presented at the Workshop of Actinomycete Taxonomy, Society for Industrial Microbiology, Houston, Texas, August 12-13, 1978.

NAKAMURA, L. K. AND C. D. CROWELL. Starch-Hydrolyzing Lactobacillus from Swine Waste-Corn Fermentations. Presented at the Society for Industrial Microbiology Meeting, Houston, Texas, August 1978.

PRIDHAM, T. G. Useful Reference Works concerned with Streptomyces and Streptoverticillia. Three page multilith for distribution to interested parties.

PRIDHAM, T. G. Micro-Organism Culture Collections: Acronyms and Abbreviations. ARS-NC-17. Additions through December 1978, 12 pp.

PRIDHAM, T. G. Report on Status of Approved List of Names of Streptomyces and Streptoverticillia. Presented at the XII International Congress of Microbiology, Intersectional Meeting, Munich, Germany, September 3-8, 1978.

PRIDHAM, T. G. Characterization and Taxonomy of Streptomyces. Presented at the Workshop on Actinomycete Taxonomy, Society for Industrial Microbiology, Houston, Texas, August 12, 1978.



PRIDHAM, T. G. Methodologies for Streptomyces and Streptovorticillia: Whole-Cell Hydrolyzate Analyses, Chromogenicity, Morphology, Color, Chromatography, Keys, Unknown. Presented at the Workshop on Actinomycete Taxonomy, Society for Industrial Microbiology, Houston, Texas, August 12-13, 1978.

SKERMAN, V. B. D., V. MCGOWAN, L. SLY, H. LECHEVALIER, D. KELMAN, A. HAYWARD, AND T. G. PRIDHAM. Assessment of Available Type and Neotype Cultures of Organisms on the Draft Approved List of Names of Bacteria and Additional Species. pp. 1-24; 56-69. University of Queensland.

6. Effect of Immobilization Procedure and Carrier on Enzymes that Hydrolyze Cereal Food Polymers (K. L. Smiley)

- a. Specific Objective: Prepare unique dextran digests, using immobilized glucanases that will be of value to food and pharmaceutical processors.

Progress: A viscometric assay for dextranase was developed that could be used on both soluble and immobilized dextranases. This assay was more sensitive for the detection of small amounts of endo-acting enzyme than the reducing sugar method commonly used. An homologous series of isomalto-oligosaccharides was prepared ranging from degree of polymerization-2 to degree of polymerization-14 by the reaction of dextransucrase on sucrose in the presence of glucose serving as a primer. The individual oligosaccharides were isolated by preparative high pressure liquid chromatography. Methylation analysis confirmed the chain length and linearity of each fraction. Dextranases from Penicillium funiculosum NRRL 1768 and P. lilacinum NRRL 896 were isolated and partially purified by conventional techniques. The purified enzymes were immobilized on phenol-formaldehyde resin and porous silica. Both carriers adsorbed significant amounts of soluble enzyme; however, effectiveness of the bound enzymes was greatly reduced when compared to the soluble forms. Even so, enough dextranase activity was present to permit meaningful studies. It was found that when dextran T-2000, a uniformly sized dextran of about  $2 \times 10^6$  MW, was used as a substrate, the viscosity of the solution decreased and reducing sugar was formed. Upon removal of the immobilized enzyme from contact with the solution, viscosity and reducing power remained constant. Therefore, no soluble enzyme was leaching from the carrier. When high molecular weight dextran B-512 was the substrate, the digests, after removal of the immobilized enzyme, continued to decrease in viscosity and reducing sugars continued to be formed (an indication that soluble enzyme was being displaced from the carrier). Repeated washing of the immobilized dextranase with dextran B-512 solutions has not resulted in a stable immobilized enzyme preparation. The reason why the high molecular weight dextran desorbs the enzyme from the carriers is not clear. An analogous situation was noticed in earlier work with immobilized alpha-amylase. High molecular weight amylopectin strongly binds to the amylase and all "loose" amylase is removed from the carrier in the presence of amylopectin. The truly covalent-bound amylase remains and active immobilized amylase preparations are obtained. Possibly the dextranase in our preparations is held to the carrier more by adsorption



than by covalent bonding, thus allowing the more strongly adsorbing B-512 dextran to remove it from the carrier. Digests of dextran T-2000 made by immobilized dextranase from either Penicillium funiculosum or P. lilacinum differed significantly from digests prepared by the soluble enzymes. The digests from immobilized enzyme action had 10-20 fold greater increases in reducing sugar values per unit decrease in viscosity than did the soluble enzymic digests. This result indicates that the immobilized enzyme is releasing many small oligosaccharides from peripheral portions of the dextran, but leaving the internal core more or less intact. Again, this action mimics that of immobilized alpha-amylase on starch whereby there is a greater increase in reducing value for a unit decrease in starch-iodine color than is the case with soluble alpha-amylase. These results reinforce our conviction that biodepolymerases, when immobilized, may attack their substrates in a different manner than when soluble.

- b. Specific Objective: Find a mechanistic explanation for the stimulation and inhibition of barley beta-amylase by chelating and reducing agents which stimulate and ascorbic acid which inhibits. Compare barley beta-amylase with the corresponding bacterial amylase regarding compounds that affect their reaction rates on starch.

Progress: The effect of stimulators and inhibitors on barley beta-amylase activity was found to be markedly influenced by pH. For instance, ascorbic acid, long known to be an inhibitor of beta-amylase, was much more effective at pH 7.0 than at pH 4.5. Likewise, at pH 7.0 sulfydryl and other reducing agents, ethylenediaminetetraacetic acid, certain organic and amino acids stimulate beta-amylase action. At pH 4.5, activation by these compounds is marginal or non-existent. These observations are physiologically significant. The optimum pH of barley beta-amylase is considered to be around 4.5. In germinating barley, the pH is closer to 6.4. Consequently, metabolic compounds present or produced during germination that might have an effect on the enzyme will exhibit a maximum effect.

Explanations as to how certain reagents stimulate or inhibit beta-amylase have been found. Ascorbic acid inhibits because it reduces any cupric ion present to the highly toxic cuprous form. Cysteine and other reducing agents stimulate because, while they reduce cupric ion to cuprous, they, unlike ascorbic acid, can chelate the copper and remove it from the enzymic reaction site. Sodium hydrosulfite, although not a chelating reagent does overcome ascorbate inhibition. We find the reason for this is due to its ability to reduce cuprous copper to metallic copper. Metallic copper is not inhibitory. It was also found that low molecular weight aldehydes, especially d-glyceraldehyde, was highly inhibitory at pH 7.0 but showed no inhibition at pH 4.35. Inhibition of beta-amylase by glyceraldehyde cannot be reversed by chelating agents, but is reversed by calcium ions. Calcium ions have no effect on ascorbate inhibition. In addition, the inhibitory constant for glyceraldehyde is about 600 times greater than the constant for ascorbate. The exact way in which the aldehyde inhibits beta-amylase is not known, but it may be

because of interaction of the aldehyde with the enzyme protein at or near the active site.

Evidence was obtained that the active form of beta-amylase involves the presence of disulfide bonds. When beta-amylase was reacted with dithiodinitrobenzoic acid (DTNB) little color formation was noticed, indicating that few free sulphydryl groups were present. Ascorbic acid by itself will reduce DTNB somewhat, but a combination of ascorbate and beta-amylase caused a large reduction of DTNB as evidenced by the large amount of color formed. Obviously, the inhibited form of barley beta-amylase contains more free sulphydryl groups than the native enzyme.

#### Publications:

HOFREITER, B. T., K. L. SMILEY, J. A. BOUNDY. Preparation of Retrogradation Resistant Starches with Immobilized Amylases. U.S. Patent 4,121,974, October 24, 1978.

HOFREITER, B. T., K. L. SMILEY, J. A. BOUNDY, C. L. SWANSON, AND R. J. FECHT. Novel Modification of Corn Starch by Immobilized alpha Amylase. Cereal Chem. 55 (1978):995-1006.

#### 7. Conversion of Cellulosic Wastes Into Feed for Ruminants (P.L. 480 Grant - Pakistan Council of Scientific and Industrial Research)

Fermented products are being fed to cattle and water buffalo; products include fermented wheat straw, bagasse pith, and rice straw. Preliminary results indicate good digestibility. Laboratory studies show that when Chaetomium and Bacillus sphaericus were used in combination on bagasse, the increase in sugar was as high as 62.5%. Increase in nitrogen was as high as 46.1% when Trichoderma and Penicillium were used together. Presumably, this represented protein made from ammonia by the molds. Levels of sugar and protein were lower in pilot-plant studies. The digestibility of pressed wheat straw was always more than the unpressed. Apparently, an increase in the size of the inoculum is important because with higher inoculum levels more biodegradation of the substrate occurred. Submerged fermentation of the substrate with Bacillus laterosporus and Streptomyces sp. resulted in 100% increase in its digestibility as compared to solid state fermentation.

#### 8. Fermentative Utilization of Cane Sugar Bagasses (P.L. 480 Grant - National Research Center, Cairo)

The work achieved in the first 6 months included (1) chemical analysis of two experimental samples of cane sugar bagasses, (2) isolation and identification of some microflora from cane sugar bagasses and (3) survey of some fungal isolates for production of cellulolytic activity. The two bagasse samples were more or less similar in their quantitative composition and contained: 2.0% ash, 37.4-41.5% cellulose, 23.7% hemicellulose, 33.4-34.7% lignin, 0.28% protein nitrogen, 0.07%



nonprotein nitrogen, 2.7-7.3% lipid materials, 1.3-6.2% gums, 0.2-1.1% fats, 0.01% sterols, and 0.4-0.9% low-molecular weight carbohydrates. No pectin was found in either bagasse samples. Acid hydrolysis of each of the bagasse samples, followed by paper chromatography of the hydrolysis products, afforded glucose (major), arabinose, xylose, and rhamnose (minor) residues. Both bagasse samples were also similar in their qualitative composition of free and bound amino acids. A total of 139 fungal, 102 bacterial, 8 yeast, and 5 isolates of actinomycetes were isolated from the investigated cane sugar bagasses. Of the bacterial isolates, 17 were identified. Of the fungal isolates, 46 were identified to the genus. Survey of 61 of the fungal isolates (including those identified) for production of cellulolytic activity was done by measuring the reduction in viscosity of buffered sodium-carboxymethyl-cellulose solution and by determination of the released reducing sugars brought about by the action of the culture filtrate. Generally, no consistent relationships seemed to exist between cellulolytic activity and the protein content of the culture filtrate or mycelial growth. Production and extent of cellulolytic activity depended on the fungal isolate and the type of culturing. The potent cultures for producing active cellulolytic enzymes included isolates of Aspergillus, Penicillium, Fusarium, Trichoderma, Gliocladium, and Rhizopus.

9. Thermophilic Microbial Conversion of Cellulosic Materials to Animal Feed  
(P.L. 480 Grant - Institute of Food Technology, Poznan)

Forty-two strains of thermophilic fungi were isolated from natural sources--composts, wood wastes--during the report period. Preliminary studies confirmed that some strains grew on native ligno-cellulosic materials. The strains of bacteria and fungi isolated in first step of project were able to grow on solid and liquid media containing filter paper or straw. The cultivations of bacteria were carried out on solid media of 75% moisture content at 50 C and in liquid media containing straw particles in suspension. Cellulase activity, level of reducing sugars and soluble protein were estimated in culture samples. Investigators have studied one-third of isolated bacterial cultures. They have confirmed that these bacteria in spite of growth on cellulosic material show very low cellulolytic activity. Fungi were cultivated at 52 C on solid media of 85% of moisture content prepared from wheat straw enriched in mineral salts. It was found that ca. 15% from among 85 investigated strains of fungi showed good growth and development on cellulose-rich media. The utilization of cellulose has reached about 30%.

D. TECHNOLOGIES FOR FOOD AND FEED  
USES - ANIMAL PRODUCTS

1. Conversion of Feedlot Wastes into Feed Supplements by Fermentation with Grain (G. R. Hrubant)

- a. Specific Objective: Continue study of semicontinuous batch fermentation of fresh swine waste combined with corn. Examine effect of



increased time of batch fermentation or of adding whey to kill coliforms during the fermentation.

Progress: Ten-percent portions of fermentation product from fresh swine waste-corn cultures were used serially as inoculum at 28°C for four successive batch cultures incubated aerobically for 72 hr and for 17 successive batch cultures incubated for 96 hr. Lactic acid bacteria in both feedback intervals increased 100-fold in 24 hr from initial inoculum flask levels of  $10^7$ /dry g. These results are comparable to that found with 48-hr feedback cycles. The effect on microbial growth patterns of feeding back portions of previous cultures to succeeding fermentations was to make lactic acid bacteria the dominant group of microorganisms at the beginning and throughout each cycle. The effect of variation in feedback cycle time on the growth pattern of fecal coliforms did not include consistent cyclic disappearance of this group with pH levels near 4. Feedback cycles of 72 hr gave coliform levels of  $10^5$ /dry g with pH near 4 but disappearance of coliforms in two instances was also associated with this pH level. Similar results were found with the 96-hr feedback mode, coliform removal associated with pH 6.10 in one instance and pH 4.27 in the other instance. The remaining cycles in the 96-hr mode contained coliform bacteria at levels of  $10^7$  to  $10^6$ /dry g and some cycles had pH levels near 4. Yeast cells showed a growth pattern of decreasing numbers within 72- and 96-hr feedback cycles, comparable to the 48-hr mode. Instances of yeast cell proliferation were associated with increases in pH, usually above 5. Increasing the fermentation time of feedback from 48 to 72 and 96 hr resulted in respective total acid production of (mean  $\pm$  standard deviation)  $0.13 \pm 0.07$ ,  $0.34 \pm 0.04$ , and  $0.31 \pm 0.13$  meq/dry g to give corresponding pH values for finished feedback cycles of  $4.63 \pm 0.31$ ,  $4.36 \pm 0.18$ , and  $4.59 \pm 0.32$ . This suggests that 72-hr cycles encourage genera of lactic acid bacteria which make more acid with lower pH levels than either 48- or 96-hr modes.

- b. Specific Objective: Continue selection of those isolates from waste-grain fermentations which may increase protein-producing capacities in directed fermentations.

Progress: Subcultures of 250 antibiotic-resistant lactobacilli were screened for lysine, cystine, and methionine excretion. Sixteen excretors were reserved for further testing. One streptobacterium excreted both lysine and cystine, and five others excreted lysine only. Ten cultures (four streptobacteria, three thermobacteria, and three beta-bacteria) excreted cystine. All 16 are resistant to streptomycin, all but one to tetracycline, and 10 are also resistant to chlortetracycline, chloramphenicol, and/or penicillin. None are starch hydrolyzers.

- c. Specific Objective: Characterize starch-hydrolyzing lactobacilli isolates from previous swine waste-corn fermentations.

Progress: Starch-hydrolyzing lactobacillus isolates from previous swine waste-corn fermentations were characterized. Studies revealed

that the starch-metabolizing lactobacillus hydrolyzed starch by means of an extracellular,  $\alpha$ -amylase-like enzyme. This organism was capable of converting approximately 90% of soluble starch (initial concentration, 1%) to lactic acid in 72 hr. Analyses showed that the average guanine-plus-cytosine (G+C) content of the deoxyribonucleic acid (DNA) from six representative strains ranged from 44.0 to 45.5 mol %. Preliminary investigations indicated about 30% homology of DNA of the starch hydrolyzers with that of Lactobacillus casei. This suggested some relationship between the two organisms. Based on the completed characterizations, it has been proposed that the starch-hydrolyzing organism merited recognition as a species with the name Lactobacillus amylophilus.

- d. Specific Objective: Study the effect of inoculating the swine waste-corn fermentation with homo- and heterofermentative Lactobacillus previously isolated from a swine waste fermentation.

Progress: Inoculation of swine waste-corn mixtures with 1.5% inocula of starch-hydrolyzing lactobacilli produced fermentations where the starch hydrolyzers were the predominant Lactobacillus counted. Proliferating rapidly, the number of starch hydrolyzers increased from an initial count of  $3 \times 10^7$  to a high of  $8 \times 10^9$  bacteria/g in 24 hr. Other lactobacilli numbered about  $2.5 \times 10^9$  organisms/g at 24 hr. Although declining in numbers, the starch-hydrolyzing lactobacilli persisted in the fermentation for at least 16 days.

Although the initial pH values of inoculated swine waste-corn fermentations decreased more rapidly than those of uninoculated controls, the minimum pH values eventually obtained in 3 to 4 days in the test and control runs were the same, namely 4.5.

- e. Specific Objective: Examine effect of antibiotics in the continuous FLWL-corn fermentation.

Progress: The continuous fermentation of corn-FLWL mixtures operated normally when using FLWL from cattle which had been fed antibiotics. No differences were observed in the viability of antibiotic resistant or sensitive coliforms or lactobacilli during the fermentation. Examination of the microflora showed that the animals had been fed both aureomycin and streptomycin but were not currently on antibiotics. This was based on (1) the total numbers of organisms, (2) the proportions of the dominant aerobe, coliforms, and lactics in the population, and (3) the proportion of antibiotic-resistant organisms in each group. The dominant aerobe, a coryneform which usually accounts for 50% of the total population, represented only 1-2% of the microflora. About 3% of the coryneforms were resistant to streptomycin; none were resistant to aureomycin, penicillin, or chloramphenicol. The coliform count was also half that usually obtained. Two-thirds of the coliforms were resistant to aureomycin and 10% were resistant to streptomycin. All streptomycin-resistant isolates which were examined further were also resistant to aureomycin. No coliforms were resistant to chloramphenicol. Lactobacilli represented 1% of the total microflora,



10 times the expected count. One-third of the lactics were resistant to aureomycin. Insignificant numbers were resistant to penicillin or chloramphenicol. All of the lactics were resistant to streptomycin. However, 13 stock cultures from the Agricultural Research Culture Collection were also resistant to various levels of streptomycin.

#### Publications:

HRUBANT, G. R. AND R. W. DETROY. Composition and Fermentation of Feedlot Wastes. In Industrial Waste Treatment and Utilization Monograph, Pergamon Press, Oxford, England, in press.

NAKAMURA, L. K. AND C. D. CROWELL. Lactobacillus amylophilus, A New Starch-Hydrolyzing Species from Swine Waste-Corn Fermentation. In Developments in Industrial Microbiology, Volume 20, in press.

#### Other Reports:

HRUBANT, G. R. Composition and Fermentation of Feedlot Wastes. Invited presentation at Industrial Waste Treatment and Utilization Conference, Waterloo, Ontario, Canada, July 5-7, 1978.

NAKAMURA, L. K. Lactobacillus amylophilus, A New Starch-Hydrolyzing Species from Swine Waste-Corn Fermentation. Presented at the Society for Industrial Microbiology Meeting, Houston, Texas, August 13-18, 1978.

### E. TECHNOLOGIES FOR INDUSTRIAL USES - PLANT AND ANIMAL PRODUCTS

#### 1. Isolation of a Perfect Stage of the Yeast *Candida utilis* (A. I. Herman)

- a. Specific Objective: Examine the effect of specific plant hormones on the development and viability of the endospore-like bodies of *C. utilis*.

Progress: Three stocks of the yeast *Candida utilis* were grown in several concentrations of the plant hormones 2-4-D and IAA in both complete and minimal media to determine the effect of these compounds on the development of the aborted spore-like bodies present in the yeast. The plant hormone 2-4-D appeared to increase vacuole size in the yeasts but neither 2-4-D nor IAA influenced the development or viability of the aborted spore-like structure produced by *C. utilis*.

#### 2. Polyprenoids as Coenzymes for the Biosynthesis of Polysaccharides in Enzyme Systems (P.L. 480 Grant - Inst. of Biochemistry and Biophysics, Warsaw)

In a yeast membrane system, chain length had little effect on sugar or sugar phosphate acceptor/donor functions of semisynthetic polyprenols. Saturation of the alpha-isoprene residue also had little



effect on synthesis of mannosyl lipid. However, utilization of mannosyl lipid for mannosylation of protein had marked preference for alpha-saturation. Polyphenol phosphates resembling natural yeast dolichol phosphate were the only effective substrates for synthesis of N-Ac glucosaminylpyrophosphorylpolyphenol. Mono-N-Ac glucosaminyl lipids were glycosyl donors to protein only after conversion to more polar lipids by addition of N-Ac glucosaminyl and mannosyl residues. Acceptor/donor functions of alpha-saturated and unsaturated forms of phosphorylated C<sub>85</sub> and C<sub>55</sub> polyphenols and retinyl phosphate were tested with isolated membrane subfractions from rat liver. Specificity was observed for alpha-saturated forms as sugar acceptors from sugar nucleotides (GDP mannose most active). Rat liver microsomal fraction also catalyzed formation of dolichol palmitate from mixture of dolichols, palmitic acid, ATP, CoA, and Mg<sup>++</sup>. A model short-chain polyphenol was synthesized that has a carbonyl in the omega-residue in place of unsaturation.

#### Publications:

PALAMARCZYK, G. AND E. JANCZURA. Lipid Mediated Glycosylation in Yeast Nuclear Membranes. FEBS Lett. 77 (1977):169-172.

MANKOWSKI, T., W. SASAK, E. JANCZURA, AND T. CHOJNACKI. Specificity of Polyphenol Phosphates in the in vitro Formation of Lipid-Linked Sugars. Arch. Biochem. Biophys. 181 (1977):393-401.

SASAK, W. AND T. CHOJNACKI. The Identification of Lipid Acceptor and the Biosynthesis of Lipid-Linked Glucose in Bacillus stearothermophilus. Arch. Biochem. Biophys. 181 (1977):402-410.

CHOJNACKI, T. Polyphenols: Structure and Involvement in Glycoprotein Synthesis. Hoppe-Seyler's Z. Physiol. Chem. 358 (1977):224.

#### Other Reports:

PALAMARCZYK, C., D. D. PLESS, A. RADOMINSKA-PYREK, T. MANKOWSKI, AND T. CHOJNACKI. Comparison of Polyphenyl Derivatives in Yeast Glycosyl Transfer Reactions. IXth International Symposium on Carbohydrate Chemistry, London, April 10-14, 1978.

### F. TECHNOLOGIES AND PRODUCTS TO INCREASE EXPORTS OF AGRICULTURAL PRODUCTS

#### 1. Soybean Food of the Traditional Oriental Type for the Export Market (H. L. Wang)

- a. Specific Objective: Investigate in situ heat-resistant bacteria spores in soybeans.

Progress: In soybean food processing, the presence of heat-resistant bacterial spores has led to problems. To screen for heat-resistant

spore-forming bacteria in soybeans grown under a range of environmental conditions, samples representing 1978 crops were collected from north central Illinois, northeastern Ohio, western Texas, Georgia, and Arkansas. Sixteen varieties of Illinois beans and five varieties of Ohio beans have been screened for total bacterial counts on surface washings as well as internal counts on surface-sterilized beans. With the exception of one variety, the Ohio bean had lower total bacteria counts than most of the Illinois beans. From those beans which showed evidence of internal contamination with spore-forming bacteria, isolates were taken and will be identified by taxonomic studies.

- b. Specific Objective: Evaluate conditions of soaking and cooking in preparing traditional soybean foods.

Progress: Traditionally, soybeans are thoroughly soaked in water before processing for any soybean foods. Recently, dilute lactic acid and sodium bicarbonate have been suggested for soaking the beans, either to reduce the bacterial growth during soaking or to improve the flavor. In this study, we found that acid- or alkaline-soaked beans possess the taste of the soaking agent. Also, the microorganisms used in soybean fermentation do not grow well on those beans. Furthermore, the protein solubility of acid-soaked beans is greatly reduced. Therefore, the evaluation of soaking conditions and the effect of soaking on solids losses and cooking quality was only carried out with water. Air-dried soybeans absorb water rapidly for the first 2 hours, followed by a slower rate of uptake. The beans take up an equal weight of water after approximately 2.5 hours at 2 X 37°C to 5.5 hours at 20°C and reach complete hydration (2.4 times weight) after 6 hours at 37°C. Soluble solids are leached out of the beans at a fairly steady rate throughout the hydration and the amount is greater with higher temperatures; 5% solids loss after 24 hours at 20°C to 10.5% at 37°C. Of the total solids lost, 7-16% is protein. The proportion of protein loss increases as the soaking time and temperature increase. About 30-50% of soluble sugars, including fructose, sucrose, raffinose, and stachyose, are removed from the beans after overnight soaking at 25°C. On the other hand, the amounts of trypsin inhibitor and hemagglutinin found in the soybean soak are relatively small as compared to that present in the air-dried beans. Hydrating soybeans to double the weight prior to cooking reduces the cooking time, increases the tenderness, and improves their appearance. Complete hydration results in no further improvement in cooking rate or cooking quality of the beans except that the weight of the cooked beans is increased.

#### Publications:

WANG, H. L., G. C. MUSTAKAS, W. J. WOLF, L. C. WANG, C. W. HESSELTINE, AND E. B. BAGLEY. Soybeans as Human Food. URR No. 5, 54 pages, SEA, USDA, January 1979.

WANG, H. L., E. W. SWAIN, C. W. HESSELTINE, AND M. R. GUMBMANN.  
Protein Quality of Wild Rice. J. Agric. Food Chem. 26 (1978):309-312.

SWAIN, E. W., H. L. WANG, AND C. W. HESSELTINE. Note on Vitamins and Minerals of Wild Rice. Cereal Chem. 55 (1978):412-414.

HESSELTINE, C. W. Some Important Fermented Foods of Mid-Asia, the Middle East, and Africa. In press, J. Am. Oil Chem. Soc.

HESSELTINE, C. W. AND H. L. WANG. Fermented Foods. Submitted, Chem. Ind.

#### Other Reports:

HESSELTINE, C. W. Thirty Years of Adventure with the Fungi. Presented at Illinois Society for Microbiology Pasteur Award Meeting, Chicago, Illinois, October 14, 1978.

HESSELTINE, C. W. Some Important Fermented Foods of the Near East and Africa. Presented at the World Conference on Vegetable Food Proteins, Amsterdam, The Netherlands, October 29-November 3, 1978.

HESSELTINE, C. W. Some important fermented foods of the Near East and Africa. Presented at round table of World Conference on Vegetable Food Proteins, Amsterdam, The Netherlands, October 29-November 3, 1978.

HESSELTINE, C. W. Fermented foods. Presented at Agricultural University, Wageningen, The Netherlands, November 9, 1978.

HESSELTINE, C. W. Fermented Foods. Presented at the Society of Chemical Industry Symposium on Fermented Foods Meeting, London, England, November 20-24, 1978.

HESSELTINE, C. W. Fermented Foods. Presented at the University, Reading, England, November 23, 1978.

### G. NATURAL TOXICANTS AND MICROBIAL TOXINS

#### 1. Studies on Mycotoxins in Cereal Grains and Their Control (C. W. Hesseltine and O. L. Shotwell)

- a. Specific Objective: Develop analytical methods (screening and quantitative) for zearalenone that can be used on other mycotoxins and substances such as diethylstilbesterol that may also be found in agricultural commodities.

Progress: A screening method utilizing minicolumns has been applied to zearalenone and diethylstilbesterol. Diethylstilbesterol has been irradiated to attain a fluorescence that could be detected at lower



levels on TLC plates or minicolumns. Smaller plates than usual or microscope slides have been used to decrease analysis time. There was participation in a second collaborative study with the University of Minnesota on a GC method to determine zearalenone in agricultural commodities.

- b. Specific Objective: Continue survey of wheat and corn in Virginia to determine yearly changes in mycotoxin levels and incidences (zearalenone, aflatoxin, and ochratoxin). Survey corn from Georgia, North Carolina, Texas, Kansas, Kentucky, Missouri, and Virginia to determine by random sampling definite aflatoxin levels and incidences.

Progress: In the continuing study of Virginia wheat and corn, no aflatoxin, ochratoxin, or zearalenone were found in 1978 wheat. No zearalenone or ochratoxin were found in 1978 corn, but there was a 26% incidence of aflatoxin  $\geq 20$  ppb. The Statistical Reporting Service, USDA, contributed 1,174 samples of 1978 corn used to predict crop yields collected in 12 states. The samples were analyzed after examination for Aspergillus flavus and the bright greenish-yellow fluorescence (BGY) associated with A. flavus. For comparison, two methods of inspecting corn for BGY were used on 248 of these samples. In the first, whole kernel corn was examined in an apparatus designed so a monolayer of corn on a vibrating tray can be inspected under ultraviolet light (365 nm). In the second method, coarse-ground corn was examined under UV as it came from the disc mill. The same results were obtained by both methods. Aflatoxin was not found in levels of  $> 20$  ppb in Illinois, Indiana, Iowa, Kentucky, Nebraska, or Ohio. Three percent of the samples assayed from Kansas and from Missouri had  $> 20$  ppb aflatoxin. Aflatoxin was found in incidences and levels that might cause concern in Georgia (64%  $\geq 20$  ppb), North Carolina (30%  $\geq 20$  ppb), Texas (14%  $\geq 20$  ppb), and Virginia (10%  $\geq 20$  ppb). A survey was made of the A. flavus contamination and the aflatoxin content of 238 corn samples collected during the 1977 crop year from North Carolina by J. W. Dickens, SEA-AR, Raleigh. Fifty-nine percent of the samples contained aflatoxin over 20 ppb. The amount of A. flavus internal contamination in the samples ranged from 2 to 44% on the basis of counties. The amount of infection of A. flavus correlated well with the total aflatoxin. The amount of Penicillium funiculosum and A. niger was quite high with the percent infection of P. funiculosum ranging from less than 3% to 30% and A. niger from 1% to as high as 37%.

- c. Specific Objective: Find corn hybrids resistant to zearalenone formation and to study the formation of zearalenone in the field.

Progress: Field work in cooperation with the University of Illinois on ear rot, Fusaria invasion, and zearalenone formed was completed. There was a positive relationship between amount of ear rot and zearalenone formed.

- d. Specific Objective: Evaluate rapid quantitative methods for aflatoxin  $M_1$  in dairy products and study the preparation and stability of  $M_1$  standards.

Progress: The development of a rapid method for determining  $M_1$  in dairy products has been completed. The method is currently being

evaluated in a joint AOAC-IUPAC collaborative study. Preliminary examination of data indicate the method is accurate and precise; however, minor modifications will be recommended for improvement. Acetonitrile:benzene (1+9) has been studied as a solvent system for preparing  $M_1$  standard solutions. No extraneous fluorescent zones have been detected in solutions sealed in glass ampules after 10 months' storage. Ultraviolet spectrophotometry of stored solutions (10  $\mu\text{g/ml}$ ) reveal a standard deviation of  $\pm 0.002$  absorbance units (coefficient of variation of 0.37%). Thin-layer chromatography (TLC) and densitometry of powdered milk extracts dissolved in chloroform and in acetonitrile:benzene (1+9) gave no measurable differences between the two solvents.

- e. Specific Objective: Determine the incidence of zearalenone in Fusaria-damaged 1977 Midwest corn.

Progress: Zearalenone was not detected in 62 of the 1977 corn samples with the highest amount of Fusaria damage. The detection limit of the assay was 200 ppb zearalenone.

- f. Specific Objective: Detoxification of zearalenone.

Progress: Post-alcoholic-fermentation solids from zearalenone-contaminated corn were treated with aqueous formaldehyde and then dried. During the drying process (50°C or more), zearalenone is destroyed. Aqueous formaldehyde was added (1) to stillage after ethanol was removed with steam, and (2) to wet solids recovered from filtered stillage. After water was removed from stillage and wet solids, no zearalenone could be detected by TLC, gas chromatography (GC), or gas chromatography-mass spectroscopy (GC-MS). In a preliminary test, swine did not refuse to eat corn that had been treated with paraformaldehyde.

- g. Specific Objective: Rapid screening methods to detect aflatoxin in corn will be evaluated.

Progress: A collaborative study of three minicolumn screening tests for aflatoxin in corn showed that the limit of detection for all three methods was 10 ppb aflatoxin in all laboratories. More experienced analysts could detect 5 ppb. As a result of the study, the method combining the Holaday extraction and cleanup and the Velasco column was adopted in official first action by the Association of Official Analytical Chemists.

- h. Specific Objective: Determine whether aflatoxin is found in the ears, husks, stalks, and leaves of contaminated corn plants that might be used for forage.

Progress: Fifty corn plants collected in a field where the harvested corn contained 1,000 ppb aflatoxin were studied. Plants were separated into kernels, cobs, husks, stalks, and leaves. Aflatoxin was detected in the kernels of 42 plants in levels of 2-18,300 ppb. Very



little aflatoxin was detected in the cobs. Cobs are used in xylitol production and mushroom culture. Even less toxin was found in husks, stalks, and leaves.

- i. Specific Objective: Simplify the gaseous ammonia process for detoxifying aflatoxin-contaminated corn.

Progress: It was demonstrated on a pilot-plant and farm scale that the NRRC ammonia process for detoxifying aflatoxin corn could be simplified by eliminating the ammonia recycling step of the process. Pilot-plant tests (14 bushels) showed that reduction in aflatoxin B<sub>1</sub> from 100 ppb to 9 ppb occurred after 24 hr when 1% ammonia was used in the bin with no recycling. After 9 days, B<sub>1</sub> was not detectable. In the control bin (4-hr recycle), aflatoxin was reduced to 25 ppb after 24 hr and 3 ppb after 9 days. In a test at Trivoli, Illinois, 325 bushels of corn (19.5% moisture) was treated with 0.8% ammonia in a nonrecycle system, with aflatoxin contents being reduced from 266 ppb to nondetectable after 6 days. In another test, about 400 bushels of corn containing 329 ppb aflatoxin was treated in a pile on the ground, while contained under a plastic tent. About 1.5% ammonia was added with the result that B<sub>1</sub> had been reduced to nondetectable levels after 2 weeks. Some problems in the distribution of ammonia appeared and will warrant further study.

- j. Specific Objective: Prepare material for duck-feeding studies.

Progress: Cooperative work was undertaken with Cornell University Duck Research Laboratory, Eastport, L.I., New York, to investigate preliminary aflatoxin levels which cause acute and chronic systems of aflatoxicosis in ducks. Ducklings fed a ration containing 155 ppb aflatoxin B<sub>1</sub> per kilogram of feed showed 57% less weight gain and a 75% mortality rate after 21 days as compared to the uncontaminated control.

- k. Specific Objective: Acquire basic information on procedures required for development of interregional test plots for early warning of aflatoxin in preharvest corn.

Progress: Field experiments were performed to provide information on the feasibility of developing a system for early detection of potential aflatoxin contamination in preharvest corn. A commercial hybrid adapted for growth in the South and a hybrid adapted to the Corn Belt were grown at nine diverse locations in the United States. Incidence of aflatoxin during ear development ranged from zero in the Corn Belt samples to 75% in Florida test corn, with no pattern of hybrid difference in toxin occurrence. Incubation of test ears for 7-10 days at 28°C immediately after harvest did not change toxin incidence significantly. Inoculation of Missouri test ears with *A. flavus* Link ex Fr. spores 20 days after flowering provided accumulation of 997 ppb of aflatoxin B<sub>1</sub> 20 days later. Inoculated ears of the regionally nonadapted variety exhibited higher aflatoxin levels than did corn from the adapted hybrid. Weather data from the test



locations provided preliminary evidence for association between temperature-precipitation during corn development and the extent of aflatoxin occurrence. Early estimation of aflatoxin in field corn appears to require evaluation of several environmental factors that affect the interaction between developing kernels and corn predators. In another study, 28 possible single crosses of corn from 8 randomly selected inbred lines were examined for aflatoxin B<sub>1</sub> production. Ears were artificially inoculated with conidia of A. flavus. In a diallel analysis of the aflatoxin B<sub>1</sub> data, highly significant general combining ability effects were found but the specific combining ability effects were found to be nonsignificant. The results suggest that the levels of aflatoxin B<sub>1</sub> observed in corn infected with A. flavus were under genetic control.

1. Specific Objective: Bioproduction of <sup>14</sup>C ochratoxin A in submerged culture.

Progress: A number of Aspergillus and Penicillium species were tested for production of ochratoxin A in several media. After 8 days of static incubations of submerged cultures at 28°C, toxin yields of 25 and 30 µg/ml were obtained with A. alliaceus NRRL 4181 in Ferreirás and 2% yeast extract-4% sucrose media, respectively. However, the largest production observed in the preliminary screening was 54 µg/ml; this highest level was produced by A. sulphureus NRRL 4077 in a modified Czapek solution. The medium contained the basal salts and sucrose of Czapek plus urea (3%) and corn steep liquor (0.5% solids). A time study of toxin production demonstrated maximum yield of 350 µg/ml by the A. sulphureus isolate in the modified Czapek medium after 11 days of static incubation at 28°C. The optimal production conditions were employed in additional tests designed to measure the efficiency of <sup>14</sup>C incorporation from sodium (1-<sup>14</sup>C)-acetate into ochratoxin. Samples (20 µCi) of sodium acetate were added to separate culture flasks at 24-hr intervals during the initial 9 days of the fermentation. Addition of (<sup>14</sup>C)acetate on day 4 of incubation provided the maximum yield of labeled ochratoxin. The highest specific activity of labeled toxin obtained was 0.07 µCi/mg of ochratoxin and the maximum incorporation rate of labeled acetate was 5.3%.

- m. Specific Objective: Determine the fate of Fusarium toxins during ethanol fermentation utilizing contaminated corn.

Progress: Two lots of contaminated corn were used as substrate for ethanol fermentation. One lot was naturally contaminated with zearalenone (8 ppm), and a second lot of field-inoculated corn was contaminated with zearalenone (5 ppm) and deoxynivalenol (15 ppm). Conditions routinely used in commercial ethanol fermentation were employed in bench-scale (50-liter) experiments. Ethanol production from damaged corn was comparable to production from sound corn (7-9%). Ethanol produced from contaminated corn was free of toxins. However, post-fermentation solids were contaminated and no significant reduction in zearalenone or deoxynivalenol occurred during the fermentation process. Corn naturally contaminated with aflatoxin was

utilized as a substrate in conventional ethanol fermentation. Distribution of toxin in various process and recovery fractions demonstrated no appearance of the mycotoxin in the distilled ethanol but accumulation of the toxin in spent grain. Sodium hydroxide, ammonium hydroxide, sodium hypochlorite, and hydrogen peroxide were identified as efficient agents of toxin degradation.

- n. Specific Objective: Improve production and purification procedures to obtain quantities of moniliformin necessary for chicken-feeding trials.

Progress: Procedures to produce, purify, and assay moniliformin were developed. Several grams of toxin were produced for research needs. Lethal dose ranges were determined for day-old chickens, mice, and chick embryos. In cooperation with the University of Minnesota, feeding trials are planned to determine the effect of ingested moniliformin on broiler chickens. About a gram of crystalline toxin and several kilograms of highly toxic molded substrate was provided the University of Minnesota for this study. Early trials indicate birds eating moniliformin-spiked feed show no ill effects; whereas, birds injected with small amounts of toxin (4 mg/kg body weight) die quickly.

- o. Specific Objective: Study the synergistic effects of antibiotics produced by Fusarium acuminatum NRRL 6227 on Penicillia and Aspergilli species and their synergistic effects on chick embryo or small animals.

Progress: A cyclic peptide of F. acuminatum NRRL 6227 inhibitory for Penicillium and Aspergillus species was purified. Tests with embryonating chicken eggs indicate the peptide to be nontoxic, and it did not increase the toxicity of T-2 toxin in the chick-embryo test.

- p. Specific Objective: Survey of field corn infected with Gibberella zeae for mycotoxins in Paulding, Williams, Defiance, and Fulton counties in Northwest Ohio.

Progress: Preharvest samples (52) were collected from 26 farms in the 4 counties in northwest Ohio and assayed for vomitoxin, T-2 toxin, and diacetoxyscirpenol. T-2 toxin or diacetoxyscirpenol were not detected in any of the samples, but vomitoxin was detected in 24 samples (0.5-10 ppm).

- q. Specific Objective: To determine structure of a compound isolated from A. clavatus in cooperation with Dr. A. K. Sarbhoy, Division of Mycology, Plant Pathology, Indian Agricultural Research Institute, New Delhi, India.

Progress: The metabolite from A. clavatus was isolated, purified, and identified as succinic acid. Dr. A. K. Sarbhoy has found it has weak toxic activity against certain pathogenic as well as saprophytic microorganisms.



- r. Specific Objective: To determine structure of the antibiotic equisetin.

Progress: A structure has been determined compatible with spectroscopic data and degradation products.

- s. Specific Objective: Develop fermentations for production, isolation, and purification of mycotoxins.

Progress: A method was developed to isolate multi-gram quantities of crystalline ochratoxin A from fermented wheat by liquid-liquid extraction procedures and subsequent high-pressure liquid chromatography. A 4-g quantity of crystalline ochratoxin A was sent to P. B. Hamilton, North Carolina State University, for toxicological studies with poultry. A solid substrate fermentor was designed and instrumented to allow investigation of parameters involved during production of secondary metabolites by various molds. A method was developed for production of xanthomegnin by P. viridicatum on whole rice in shaken flasks. Isolation procedures and quantitative analysis by high-pressure liquid chromatography have been determined.

- t. Specific Objective: Test the hypothesis that formation of a bright greenish-yellow fluorescence in seeds colonized by A. flavus can occur solely as a result of the activities of cooccurring microorganisms.

Progress: The detection of a bright greenish-yellow fluorescence (BGYF) under ultraviolet light (365 nm) has been associated with the presence of kojic acid-producing strains of A. flavus and A. parasiticus. Because a number of investigators failed to detect BGYF in autoclaved plant materials inoculated with these fungi, it was theorized that plant tissue enzymes were essential to the formation of BGYF. Inoculation of autoclaved corn kernels simultaneously with A. flavus (NRRL 6412) and certain individual microfungal isolates from corn (i.e., Alternaria alternata, Cladosporium cladosporioides, Curvularia lunata, Fusarium moniliforme, Penicillium variable and an unidentified yeast, followed by 8 days' incubation at 28°C, resulted in the formation of BGYF. It becomes clear from these observations that any field or storage-related microenvironment suitable for fungal biosynthesis of kojic acid and peroxidases in cereal grains will produce BGYF. Until now, microbiologists have not been able to explain how the incidence of BGYF in mature cereal grains is able to increase in lots of nongerminating seed. Generally, members of the microfungal community that did not interfere with the development of A. flavus when paired on agar plates were also those which produced a characteristic BGYF.

- u. Specific Objective: Test the hypothesis that aflatoxin biosynthesis in A. flavus is differentially affected by cooccurring fungal taxa according to their ecological status in a competitive hierarchy.

Progress: Aflatoxin levels were found to differ substantially among cultures of autoclaved corn kernels simultaneously coinoculated with



A. flavus and individual fungal isolates from corn sampled at harvest. The status of individual fungal colonists as interference competitors, and the order in which these colonists become established within the kernel (i.e., before, during, or after colonization by A. flavus), could be related to differences in aflatoxin levels.

- v. Specific Objective: Synthesize and study the reactions of aflatoxin-like model compounds with ammonia.

Progress: Two model compounds have been synthesized and reacted with ammonia. One of these, 5,7-dimethoxycyclopentenon[2,3-C]coumarin, essentially duplicates three of the rings of aflatoxin B<sub>1</sub>. It reacts with ammonia to give a product analogous to aflatoxin D<sub>1</sub>.

- w. Specific Objective: Continue the investigation of nitrogen-containing lipids in the oil from ammoniated corn.

Progress: About one-half of the altered fatty acids (primarily linolenic acid) can be accounted for in the oxygenated or oxidized portion of the oil. The oxygenated acids are those normally associated with lipoxygenase activity. No nitrogenous lipids other than the amides previously reported were found. The high nitrogen content in the lipid appears in compounds that have carbohydrate characteristics and must have been formed by ammonia-carbohydrate reactions. These ammonia-carbohydrate reaction products were then extractable with the nonpolar solvent used in lipid extraction.

#### Publications:

BENNETT, G. A. AND R. A. ANDERSON. Distribution of Aflatoxin and/or Zearalenone in Wet-Milled Corn Products. J. Agric. Food Chem. 26 (1978):1055-1060.

BENNETT, G. A., E. E. VANDEGRAFT, O. L. SHOTWELL, S. A. WATSON, AND B. J. BOCAN. Zearalenone: Distribution in Wet-Milling Fractions from Contaminated Corn. Cereal Chem. 55 (1978):455-461.

BLACK, L. T., G. F. SPENCER, AND O. L. BREKKE. Reactions of Lipids in Corn with Ammonia. J. Am. Oil Chem. Soc. 55 (1978):526-529.

BREKKE, O. L., A. C. STRINGFELLOW, AND A. J. PEPLINSKI. Aflatoxin Inactivation in Corn by Ammonia Gas: Laboratory Trials. J. Agric. Food Chem. 26 (1978):1383-1389.

BURMEISTER, H. R., A. CIEGLER, AND R. F. VESONDER. Moniliformin, A Metabolite of Fusarium moniliforme: Production, Purification, and Toxicity. Appl. Environ. Microbiol. 37 (1979):11-13.

CALVERT, O. H., E. B. LILLEHOJ, W. F. KWOLEK, AND M. S. ZUBER. Aflatoxin B<sub>1</sub> and G<sub>1</sub> Production in Developing Zea mays Kernels from Mixed Inocula of Aspergillus flavus and A. parasiticus. Phytopathology 68 (1978):501-506.

- CIEGLER, A. Detoxification of Aflatoxin-Contaminated Agricultural Commodities. In Toxins: Animal, Plant and Microbial, Proceedings of Fifth International Symposium, San Jose, Costa Rica, August 8-13, 1976, P. Rosenberg, ed., pp. 729-738. Pergamon Press, New York. 1978.
- CIEGLER, A. Trichothecenes: Occurrence and Toxicoses. J. Food Protection 41 (1978):399-403.
- CIEGLER, A. Fungi That Produce Mycotoxins: Conditions and Occurrence. Mycopathologia, in press.
- CIEGLER, A., K. A. BURBRIDGE, J. CIEGLER, AND C. W. HESSELTINE. Evaluation of Sclerotinia sclerotiorum as a Potential Mycotoxin Producer on Soybeans. Appl. Environ. Microbiol. 36 (1978):533-535.
- CONWAY, H. F., R. A. ANDERSON, AND E. B. BAGLEY. Detoxification of Aflatoxin-Contaminated Corn by Roasting. Cereal Chem. 55 (1978):115-117.
- FENNELL, D. I., E. B. LILLEHOJ, W. F. KWOLEK, W. D. GUTHRIE, R. SHEELEY, A. N. SPARKS, N. W. WIDSTROM, AND G. L. ADAMS. Insect Larval Activity on Developing Corn Ears and Subsequent Aflatoxin Contamination of Seed. J. Econ. Entomol. 71 (1978):624-628.
- HESSELTINE, C. W. Factors Affecting Aflatoxin Production on Different Agricultural Commodities. In Proceedings of U.S. Aflatoxin Delegation Meeting, Zurich, Switzerland, March 20-21, 1978. pp. 152-165. 1978.
- HESSELTINE, C. W. Introduction, Definition, and History of Mycotoxins of Importance to Animal Production. Proceedings of Symposium on the Interaction of Mycotoxins in Animal Production, accepted.
- HESSELTINE, C. W. R. F. ROGERS, AND O. L. SHOTWELL. Fungi, Especially Gibberella zeae, and Zearalenone Occurrence in Wheat. Mycologia 70 (1978):14-18.
- JACKSON, L. K. AND A. CIEGLER. Production and Analysis of Citrinin in Corn. Appl. Environ. Microbiol. 36 (1978):408-411.
- KARKI, T. B., R. J. BOTHAST, AND R. D. STUBBLEFIELD. Microbiological and Aflatoxin Analyses of Cereal Grains from the Tarai Plain of Southern Nepal. Cereal Chem. 56 (1979)41-42.
- KROGH, P., R. ELLING, C. FRIIS, B. HALD, A. E. LARSEN, E. B. LILLEHOJ, A. MADSEN, H. P. MORTENSEN, F. RASMUSSEN, AND U. RAVNSKOV. Experimental Porcine Nephropathy Induced Perorally by Long-Term Exposure to Ochratoxin A. Acta Pathol. Microbiol., accepted.
- KWOLEK, W. F. AND O. L. SHOTWELL. Aflatoxin Occurrence in Some White Corn Under Loan. V. Aflatoxin Predictions from Weight Percent Bright Greenish-Yellow Fluorescence. Cereal Chem., in press.

- LILLEHOJ, E. B. Use of Mycotoxin Contaminated Grain in the Ethanol Fermentation. Proc. Distill. Feed Res. Council 33 (1978):23-30.
- LILLEHOJ, E. B., O. AALUND, AND B. HALD. Bioproduction of [ $^{14}\text{C}$ ] Ochratoxin A in Submerged Culture. Appl. Environ. Microbiol. 36 (1978):720-723.
- LILLEHOJ, E. B., D. I. FENNELL, W. F. KWOLEK, G. L. ADAMS, M. S. ZUBER, E. S. HORNER, N. W. WIDSTROM, H. WARREN, W. D. GUTHRIE, D. B. SAUER, W. R. FINDLEY, A. MANWILLER, L. M. JOSEPHSON, AND B. J. BOCKHOLT. Aflatoxin Contamination of Corn Before Harvest: Aspergillus flavus Association with Insects Collected from Developing Ears. Crop Sci., in press.
- LILLEHOJ, E. B., W. F. KWOLEK, F. ELLING, AND P. KROGH. Tissue Distribution of Radioactivity from Ochratoxin A- $^{14}\text{C}$  in Rats. Mycopathologia, accepted.
- LILLEHOJ, E. B., W. F. KWOLEK, M. S. ZUBER, O. H. CALVERT, E. S. HORNER, N. W. WIDSTROM, W. D. GUTHRIE, G. E. SCOTT, D. L. THOMPSON, W. R. FINDLEY, AND A. J. BOCKHOLT. Aflatoxin Contamination of Field Corn: Evaluation of Regional Test Plots for Early Detection. Cereal Chem. 55 (1978):1007-1013.
- NOFSINGER, G. W. AND R. A. ANDERSON. A Note on Inactivation of Aflatoxin in Ammonia-Treated Shelled Corn at Low Temperatures. Cereal Chem., accepted.
- PETERSON, R. E. AND A. CIEGLER. Ochratoxin A: Isolation and Subsequent Purification by High Pressure Liquid Chromatography. Appl. Environ. Microbiol. 36 (1978):613-614.
- RICHARD, J. L., J. R. THURSTON, E. B. LILLEHOJ, S. J. CYSEWSKI, AND G. D. BOOTH. Complement Activity, Serum Protein, and Hepatic Changes in Guinea Pigs Given Sterigmatocystin or Aflatoxin, Alone or in Combination. Am. J. Vet. Res. 39 (1978):163-166.
- SHANNON, G. M. AND O. L. SHOTWELL. Collaborative Study of Three Minicolumn Methods for Aflatoxin in Yellow Corn. J. Assoc. Off. Anal. Chem., accepted.
- SHOTWELL, O. L., E. E. VANDEGRAFT, AND C. W. HESSELTINE. Aflatoxin Formation on Sixteen Soybean Varieties. J. Assoc. Off. Anal. Chem. 61 (1978):574-577.
- STUBBLEFIELD, R. D. Mycotoxins. In Densitometry in Thin Layer Chromatography: Practice and Applications, J. E. Touchstone and J. A. Sharma, eds., pp. 393-429. John Wiley & Sons, Inc., New York. 1979.
- STUBBLEFIELD, R. D. Thin Layer Chromatographic Determination of Citrinin. J. Assoc. Off. Anal. Chem. 62 (1979):201-202.



VESONDER, R. F. Xanthocillin, A Metabolite of Penicillium egyptiacum NRRL 1022. Lloydia, in press.

VESONDER, R. F., A. CIEGLER, R. F. ROGERS, K. A. BURBRIDGE, R. J. BOTHAST, AND A. H. JENSEN. Survey of 1977 Crop Year Preharvest Corn for Vomitoxin. Appl. Environ. Microbiol. 36 (1978):885-888.

WICKLOW, D. T. AND C. W. HESSELTINE. Fluorescence Formed by Aspergillus flavus in Association with Other Fungi in Steam-Sterilized Corn Kernels. Phytopathology, accepted.

ZUBER, M. S., O. H. CALVERT, W. F. KWOLEK, E. B. LILLEHOJ, AND M. KANG. Aflatoxin B<sub>1</sub> Production in an Eight-Line Diallel of Zea mays Infected with Aspergillus flavus. Phytopathology 68 (1978):1346-1349.

#### Other Reports:

BAGLEY, E. B. Decontamination of Corn Containing Aflatoxin by Treatment with Ammonia. Presented at Mycotoxin Symposium, American Oil Chemists' Society, St. Louis, Missouri, May 14-18, 1978.

BENNETT, G. A. Zearalenone in Cereal Grains. Presented at Mycotoxin Symposium, American Oil Chemists' Society, St. Louis, Missouri, May 14-18, 1978.

CIEGLER, A. Evaluation of Sclerotinia sclerotiorum as a Mycotoxin Producer on Soybeans. Presented at American Society for Microbiology Meeting, Las Vegas, Nevada, May 14-19, 1978.

CIEGLER, A. Aflatoxin. Presented at Panel on "Experiences in Controlling Exposures to Important Carcinogens in the United States," New York Academy of Sciences, New York, New York, June 21-30, 1978.

CIEGLER, A. Applied Aspects of Fungal Metabolism. Presented at Gordon Research Conferences on Fungal Metabolites: Biogenesis and Function, Plymouth, New Hampshire, July 3-7, 1978.

CIEGLER, A. Trichothecene Mycotoxins. Presented at seminar on Mycotoxins--Their Biosynthesis in Fungi, American Society for Microbiology Meeting, Las Vegas, Nevada, May 14-19, 1978.

CIEGLER, A. Mycotoxin Research at the Northern Regional Research Center: Past, Present, and Projected. Presented at Southern Regional Research Center, New Orleans, Louisiana, September 8, 1978.

HESSELTINE, C. W. Aflatoxin Survey of Corn in the Corn Belt--1977. Presented at Corn Dry Milling Conference, Northern Regional Research Center, Peoria, Illinois, May 31-June 1, 1978.

HESSELTINE, C. W. Aflatoxin Situation in 1977 Corn Crop. Presented at Food Research Institute, Madison, Wisconsin, May 4-5, 1978.

HESELTIME, C. W. Introduction, Definition, and History of Mycotoxins of Importance to Animal Agriculture. Presented at American Society of Animal Science and American Dairy Science Association Meeting, East Lansing, Michigan, July 9-13, 1978.

LILLEHOJ, E. B. Bioproduction of  $^{14}\text{C}$ -Ochratoxin A in Submerged Culture. Presented at American Society for Microbiology Meeting, Las Vegas, Nevada, May 14-19, 1978.

LILLEHOJ, E. B. Natural Occurrence of Mycotoxin in Feeds. Presented at American Society for Animal Science Meeting, East Lansing, Michigan, July 9-13, 1978.

LILLEHOJ, E. B. Toxin-Producing Fungi in Corn and Barley. Presented at Biology Colloquium, DeKalb, Illinois, September 7, 1978.

LILLEHOJ, E. B. Infection of Field Crops by Toxin-Producing Fungi. Presented at Southern Regional Research Center, New Orleans, Louisiana, September 29, 1978.

SHANNON, G. M. Collaborative Study of Three Minicolumns for Aflatoxin in Corn. Presented at Association of Official Analytical Chemists Meeting, Washington, D.C., October 15-19, 1978.

SHOTWELL, O. L., Aflatoxin Mold. Presented at Grain Elevator and Processing Society Meeting, Phoenix, Arizona, March 11-14, 1979.

SHOTWELL, O. L. BGY Fluorescence in Agricultural Commodities. Presented at Association of Official Analytical Chemists Spring Training Conference and Exposition, Atlanta, Georgia, May 1-3, 1978.

SHOTWELL, O. L. Mycotoxins--1978. Presented at Corn Dry Milling Conference, Northern Regional Research Center, Peoria, Illinois, May 31-June 1, 1978.

SHOTWELL, O. L. Survey of 1977 Midwest Corn for Aflatoxin. Presented at Association of Official Analytical Chemists Meeting, Washington, D.C., October 15-19, 1978.

SHOTWELL, O. L. Aflatoxin Distribution in Contaminated Corn Plants. Presented at Association of Official Analytical Chemists Meeting, Washington, D.C., October 15-19, 1978.

STUBBLEFIELD, R. D. The Rapid Determination of Aflatoxin  $M_1$  in Dairy Products. Presented at American Oil Chemists' Society Meeting, St. Louis, Missouri, May 14-18, 1978.

STUBBLEFIELD, R. D. Current NRRC Mycotoxin Research. Presented at NC-129 Mycotoxin Committee Meeting, Minneapolis, Minnesota, October 23-24, 1978.

VESONDER, R. F. Survey of 1977 Corn for Vomitoxin Prior to Harvest. Presented at Joint American Dairy Science Association and American Society of Animal Science Meeting, East Lansing, Michigan, July 9-13, 1978.

WICKLOW, D. T. Formation of a Bright Greenish-Yellow Fluorescence (BGY) by Aspergillus flavus in Association with Other Fungi in Steam-Sterilized Corn Kernels. Presented at American Phytopathology Society Meeting, Tempe, Arizona, October 29-November 2, 1978.

2. Products of Reactions Between Mycotoxins and Food and Feed Constituents  
(C. W. Hesseltine)

- a. Specific Objective: Study the chemistry of patulin and ochratoxin to determine the molecular basis of toxicity.

Progress: A series of 11 derivatives of patulin has been prepared, all of which retain the unsaturated lactone system of the parent toxin, but eliminate the highly reactive hemiacetal portion of the molecule. Reaction of the hemiacetal part of patulin with various derivatizing reagents gave the acetate, benzoate, thiosemicarbazone, and the syn- and anti-oxime. Other derivatives prepared include the phenylhydrazone, 2,4-dinitrophenylhydrazone, dilactone, dimethone anhydride, octahydroxanthene, and the O-methyl oxime. Chick egg embryo tests were run at 2.5 g/50-liter and 20 g/50-liter levels, using Wesson oil as a carrier wherever water would not solubilize the test compounds. Gross teratogenic effects (e.g., brain development outside skull; crossed beaks) were obtained with patulin 2,4-dinitrophenylhydrazone but not with phenylhydrazine, even though the former was not excessively toxic (30% kill). In contrast, the O-methyl oxime of patulin was less teratogenic but very toxic at the higher (20-g) concentration, even after 2 days (100% kill). Other derivatives were also toxic in varying degrees.



# HORTICULTURAL AND SPECIAL CROPS LABORATORY

L. H. Princen, Chief

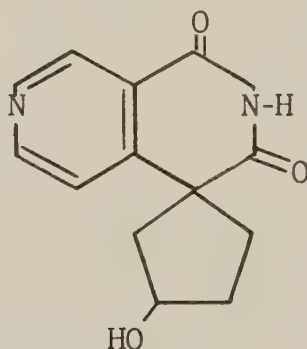
Research Leaders: R. Kleiman, J. A. Rothfus, C. R. Smith, and  
H. L. Tookey

## A. INTRODUCTION, CLASSIFICATION, MAINTENANCE, EVALUATION, AND DOCUMENTATION OF PLANT GERMPLASM

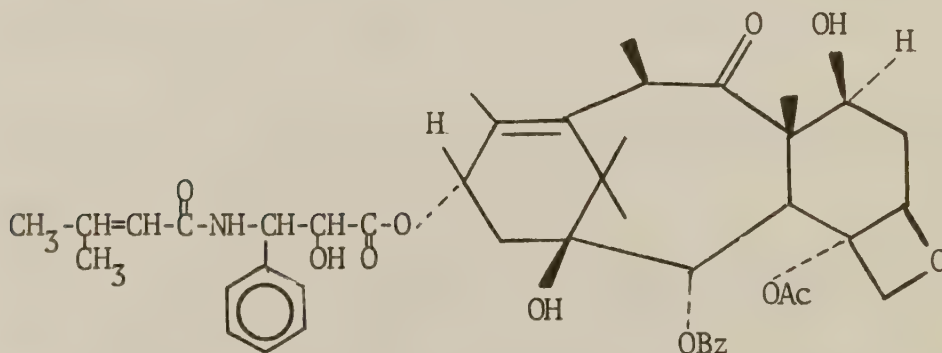
### 1. Characterization of Selected Unusual Seed Components (C. R. Smith)

- a. Specific Objective: Isolate and characterize novel constituents of plant seeds with particular attention to those with potential pesticidal value.

Progress: The structure of sesbanine, the antitumor alkaloid of Sesbania drummondii, has been determined completely and is as follows:



Structural work on a new antitumor alkaloid from Cephalotaxus mannii, cephalomannine, has been completed; it has the following structure:



Ethanol extraction of 27 kg of Trewia nudiflora seed yielded 670 g of material with both insecticidal and antitumor properties. The active principles of this extract have now been enriched approximately 27-fold.

### Publications:

POWELL, R. G., R. W. MILLER, and C. R. SMITH. Cephalomannine, a New Antitumor Alkaloid from Cephalotaxus mannii. J. Chem. Soc. Chem. Commun., in press.

POWELL, R. G., C. R. SMITH, D. WEISLEDER, D. A. MUTHARD, and J. CLARDY. A Novel Cytotoxic Alkaloid from Sesbania drummondii. J. Am. Chem. Soc. 101, in press.

## 2. Anti-Tumor Agents from Plants (C. R. Smith)

- a. Specific Objective: Develop synthetic methods that will facilitate conversion of cephalotaxine to homoharringtonine, or provide other compounds with antitumor activity.

Progress: A novel approach is being developed for attachment of the ester side chain of homoharringtonine. In principle, this approach entails two successive carbanion-mediated functionalizations at the alpha-carbon of a carboxylic acid. Favorable results have been achieved with a series of model compounds based on undecenoic acid, and most of the series of required reactions have been successfully applied to the natural ester side chain.

### Publications:

MIKOLAJCZAK, K. L. and C. R. SMITH. Synthesis of Harringtonine, a Cephalotaxus Antitumor Alkaloid. J. Org. Chem. 43 (1978):4762-4765.

KELLY, T. R., R. W. McNUTT, M. MONTURY, N. P. TOSCHES, K. L. MIKOLAJCZAK, and D. WEISLEDER. Preparation of Harringtonine from Cephalotaxine. J. Org. Chem. 44 (1979):63-67.

SMITH, C. R., K. L. MIKOLAJCZAK, and R. G. POWELL. Harringtonine and Related Cephalotaxine Esters. In Design and Synthesis of Potential Anti-Cancer Agents Based on Natural Product Models, eds., J. M. Cassady and J. D. Douros, Academic Press, New York, in press.

WEISLEDER, D., R. G. POWELL, and C. R. SMITH. Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Cephalotaxus Alkaloids. Org. Magn. Reson., in press.

## 3. Chemical Survey of Uncultivated Plants for Valuable Seed Components (R. Kleiman)

- a. Specific Objective: Discover and characterize novel plant seed compounds.

Progress: Over 400 seed samples were collected from the wild in Illinois and an additional 108 samples were received from abroad. Of these, 156 were screened for unusual chemical properties. Many exhibited unusual results from TLC, GLC, IR, and UV analyses. Of

the 112 oils converted to methyl esters and analyzed by gas chromatography, Prunus cerasoides showed 44% conjugated trienoic fatty acids, Portulaca quadrifida had 93% cyclopentenyl fatty acid (first time these acids have been found outside the Flacourtiaceae family), and a number of Compositae showed trans-3 unsaturation. Glass capillary gas chromatography has pointed to a number of heretofore unobserved unusual constituents in the methyl esters from seed oils, particularly to large amounts of cis-vaccenic acid in the Meliaceae (30% in Entandrophragma utile). Lactobacillic acid (13%) has been found for the first time as a constituent of seed oils in Byrsocarpus coccineus. Gas chromatography/mass spectrometry was used to delineate the complex combinations of acids and alcohols making up the wax esters of sperm whale oil. By using the unsaponifiables of olive oil, a gas chromatographic method for detecting adulteration of virgin olive oil with residue-B olive oil was developed. An HPLC method was developed for analysis of multiacyl triglycerides. For precise organization and accessibility, the total seed inventory and a good part of the associated chemical data have been entered into the laboratory computer.

- b. Specific Objective: Provide compositional data in support of plant breeding and agronomic research.

Progress: Chemical composition was provided on 351 samples. This includes oil percentage, fatty acid composition, and glucosinolate amounts for 139 Brassica samples for Oregon State University; oil percentage and fatty acid composition on 100 Limnanthes samples for Oregon State University; and oil and protein content in 76 Luffa samples for SEA-AR, Puerto Rico.

Conditions for extracting epoxy oil from Vernonia pauciflora seed without free fatty acid accumulation were established on a laboratory scale. Epoxy oil for evaluation (1.3 lb) was prepared; the oil (73% vernolic acid, 0.8% free fatty acid) comprised 38% of seed weight.

#### Publications:

SMITH, C. R., R. V. MADRIGAL, and R. D. PLATTNER. New Conjugated Hydroxydienoid Fatty Acids and Acetotriglycerides from Securidaca longipedunculata Seed Oil. Biochim. Biophys. Acta, in press.

SPENCER, G. F., K. PAYNE-WAHL, R. D. PLATTNER, and R. KLEIMAN. Lactobacillic and Methyl-Branched Olefinic Acids in Byrsocarpus coccineus Oil. Lipids 14 (1979):72-74.

SESSA, D. J. and R. D. PLATTNER. Novel Furaldehydes in Oxidized Soy Phospholipids. J. Agric. Food Chem., in press.

GROVE, M. D., G. F. SPENCER, P. E. PFEFFER, N. MANDAVA, J. D. WARTHEN, JR., and J. F. WORLEY. 6-D-Glucopyranosyl Fatty Acid Esters from Brassica napus Pollen. Phytochemistry 17 (1978):1187-1189.



SPENCER, G. F. and R. KLEIMAN. Palmitoleic Acid in Rourea obliquifoliata (Connaraceae) Seed Oil. J. Am. Oil Chem. Soc. 55 (1978):689.

SPENCER, G. F., and R. KLEIMAN. Detection of Spermaceti in a Hand Cream. J. Am. Oil Chem. Soc. 55 (1978):837-838.

SPENCER, G. F. Detection of B-Residue Oil in Commercial Olive Oils. J. Am. Oil Chem. Soc., in press.

SPENCER, G. F. Alkoxy-Acyl Combinations in the Wax Esters from Winterized Sperm Whale Oil by Gas Chromatography-Mass Spectrometry. J. Am. Oil Chem. Soc., in press.

BOHANNON, M. B. and R. KLEIMAN. Cyclopropene Fatty Acids of Selected Seed Oils from Bombacaceae, Malvaceae, and Sterculiaceae. Lipids 13 (1978):270-273.

HARDING, J., F. W. MARTIN, and R. KLEIMAN. Seed Protein and Oil Yields of the Winged Bean, Psophocarpus tetragonolobus, in Puerto Rico. Trop. Agric. 55 (1978):307-314.

PLATTNER, R. D. and K. L. PAYNE-WAHL. Separation of Triglycerides by Chainlength and Degree of Unsaturation on Silica HPLC Columns. Lipids, in press.

CHANG, S. P. and T. K. MIWA. Allyl Esters of Crambe-Derived Long Chain Fatty Acids and Their Fatty Polymers. J. Appl. Polym. Sci., in press.

PRINCEN, L. H. Vegetable Oil Research at NRRC for Plastics and Coatings. Coat. Plast. Prepr. 37 (1977):79-82.

PRINCEN, L. H. The Need for Renewable Coatings Raw Materials and What Could Be Available Today. J. Coat. Technol. 49 (1977):88-94.

PRINCEN, L. H. New Developments in Triglyceride Chemistry for Plastics and Coatings. In Proceedings of the Third International Conference in Organic Coatings Science and Technology, ed., A. V. Patsis and G. D. Parfitt, Technomic Publishing Company, Westport, CT, 1977, pp. 342-354.

BAKER, F. L., L. H. PRINCEN, and M. KRONSTEIN. Scanning Electron Microscopy of Marine Coatings. In Proceedings of the Fifth International Symposium on Controlled Release of Bioactive Materials, University of Akron, Akron, Ohio, 1978, pp. 748-768.

4. Natural Pest Control Agents from Seeds and Potential New Crops  
(C. R. Smith)

- a. Specific Objective: Compare sterol-steroid levels in kenaf and roselle for possible correlation with nematode resistance.

Progress: Crude extracts from kenaf and roselle were resolved poorly by chromatography on silica columns. Improved resolution with hydrolyzed extracts allowed subsequent isolation of sterols via preparative TLC. Methodology was developed for quantitation of constituents via GLC.

- b. Specific Objective: Discover and characterize useful pesticidal activity in plants.

Progress: From tomatoes resistant to the fungus Rhizoctonia solanii, a stable extract has been prepared containing the resistance factor. Partial characterization of the extract indicates probable presence of active compounds other than tomatine. A stable extract of diseased peach tree wood has been isolated which attracts female peach tree borer moths in the absence of the wood. Fractionation of the fat-soluble extract of Apium sellowianum reveals that although two well-known furocoumarins (xanthotoxin and impellatoxin) are present, these are probably not the most active compounds as judged by our assay based on treatment of European corn borer larvae.

Publication:

CARLSON, D. G. and J. A. ROTHFUS. Temperature-Based Prediction of Egg-Mass Production by Meloidogyne incognita. J. Nematol. 10 (1978): 303-307.

5. Development of Jojoba as a Crop for Arid Lands (T. K. Miwa)

- a. Specific Objective: Characterize jojoba germplasm.

Progress: Certain stored seeds show better germination than some relatively fresh seeds from different locations. Varietal differences may thus be important in seed viability. Correlations established previously between seed size/density and oil content continue as the principal basis for planting seed selection.

- b. Specific Objective: Determine properties of jojoba-derived materials and aid in jojoba technology transfer.

Progress: Solvent and solid compatibilities of jojoba oil were characterized in response to requests from growers and formulators. Beneficial synergism that imparts strength to hydrogenated jojoba-oil-polyethylene mixtures was discovered thereby.

Publications:

MIWA, T. K., G. F. SPENCER, and R. D. PLATTNER. Separation and Structure Determination of Jojoba Oil Components by High-Pressure Liquid Chromatography and Gas Chromatography/Mass Spectrometry. In Proceedings of the Second International Conference on Jojoba and Its Uses, Consejo Nacional de Ciencia y Tecnologia, Mexico City, D.F., 1978, Chapter 17, pp. 187-197.

SIMPSON, T. D. and T. K. MIWA. Crystallographic Study of Hydrogenated Jojoba Wax and Its Relationship to Polyethylene. In Proceedings of the Second International Conference on Jojoba and Its Uses, Consejo Nacional de Ciencia y Tecnologia, Mexico City, D.F., 1978, Chapter 18, pp. 199-218.

MIWA, T. K. and P. H. THOMSON. Correlation Between Density and Oil Content in Jojoba Nuts Harvested at Different Geographical Regions. In Proceedings of the Second International Conference on Jojoba and Its Uses, Consejo Nacional de Ciencia y Tecnologia, Mexico City, D.F., 1978, Chapter 19, pp. 219-228.

MIWA, T. K. and G. F. SPENCER. Composition of Jojoba Oil from Nuts Harvested at Different Geographical Regions. In Proceedings of the Second International Conference on Jojoba and Its Uses, Consejo Nacional de Ciencia y Tecnologia, Mexico City, D.F., 1978, Chapter 20, pp. 229-243.

MIWA, T. K. and J. W. HAGEMANN. Physical and Chemical Properties of Jojoba Liquid and Solid Waxes. In Proceedings of the Second International Conference on Jojoba and Its Uses, Consejo Nacional de Ciencia y Tecnologia, Mexico City, D.F., 1978, Chapter 21, pp. 245-252.

MIWA, T. K. and J. A. ROTHFUS. Sulfurized Jojoba Oil as Extreme-Pressure Lubricant. In Proceedings of the Second International Conference on Jojoba and Its Uses, Consejo Nacional de Ciencia y Tecnologia, Mexico City, D.F., 1978, Chapter 22, pp. 253-264.

MIWA, T. K. Hardness Test for Wax Formulations from Jojoba Wax, Paraffin, Polyethylene and Polypropylene. In Proceedings of the Second International Conference on Jojoba and Its Uses, Consejo Nacional de Ciencia y Tecnologia, Mexico City, D.F., 1978, Chapter 23, pp. 265-274.

MIWA, T. K. Recent Chemical Research on Jojoba and Its Uses. Yukagaku (Japan Oil Chemists' Society) 27 (1978):650-658 (in Japanese).

## B. PHYSIOLOGICAL AND BIOCHEMICAL TECHNOLOGY TO IMPROVE CROP PRODUCTION

### 1. Plant Cell and Tissue Culture for the Bioproduction of Valuable Chemicals (N. E. Delfel)

- a. Specific Objective: Determine optimum harvest time for field-grown Cephalotaxus trees.

Progress: Samples taken at seven different times from five trees at two locations under analysis.



- b. Specific Objective: Improve or simplify culture medium for C. harringtonia callus growth and alkaloid production.

Progress: Optimum culture conditions were established by multiple-interaction studies. Ammonium nitrate increases growth only if other medium components are optimal. Of factors analyzed, ammonium nitrate has greatest effect on alkaloid metabolism. Some cell lines are now autotrophic for vitamins and growth hormones. Few such lines have been reported previously.

- c. Specific Objective: Develop techniques for protoplast production.

Progress: Protoplasts were successfully prepared from Daucus carota, Pastinaca sativa, and C. harringtonia. Variety, growth conditions, or physiological state were more important than variations in technique.

- d. Specific Objective: Study possible use of fluoro-immunochemical techniques to characterize individual protoplasts.

Progress: Anti-carrot anti-serum from rabbits challenged with D. carota protoplasts cross react with parsnip and Cephalotaxus protoplasts.

- e. Specific Objective: Investigate alkaloid biosynthesis in C. harringtonia cell and tissue culture.

Progress: C. harringtonia callus can adopt an altered pattern of biosynthesis producing at least three new alkaloids. Stressed laboratory plants exhibit a similar change. Ammonium nitrate deprivation, likewise, causes a shift, but to produce yet different alkaloids.

#### Publications:

DELFEL, N. E. AND J. A. ROTHFUS. Production of homodeoxyharringtonine and Other Cephalotaxine Esters by Tissue Culture. U.S. Patent Application, Serial No. 840,423.

#### Other Reports:

DELFEL, N. E. Plant Tissue Culture--The Jolly Green Giant in a Test Tube. ACS Seminar, Peoria, Illinois, January 25, 1978.

DELFEL, N. E. Plant Cell and Tissue Culture Studies at NRRC. Public Lecture. Lakeview Museum Science Exhibit, Peoria, Illinois, February 12, 1978.

DELFEL, N. E. Tissue Culture Techniques. Overview Lecture, ARS Tissue Culture Workshop, Knoxville, Tennessee, April 19-21, 1978.

DELFEL, N. E. AND LESLIE J. SMITH. The Importance of Media Component Interactions on Plant Tissue Culture Growth. Paper presented at the Illinois Academy of Science Meeting, Normal, Illinois, April 21-22, 1978.

SMITH, LESLIE J., HELEN J. GASDORF, AND N. E. DELFEL. Plant Tissue Culture Research at NRRC. Manned exhibit for Sigma Xi Science Exhibit, Peoria, Illinois, May 6-7, 1978.

DELFEL, N. E. Plant Tissue Culture and the Production of Antitumor Alkaloids. Seminar at St. Francis Hospital, Peoria, Illinois, June 6, 1978.

DELFEL, N. E. Growth and Alkaloid Metabolism in Cephalotaxus harringtonia Tissues. Paper presented at the Fourth International Congress of Plant Tissue and Cell Culture, Calgary, Canada, August 20-25, 1978.

DELFEL, N. E. Plant Tissue Culture and the Production of Secondary Metabolites. USDA Metabolism and Radiation Laboratory, Fargo, North Dakota, August 28, 1978.

#### C. AGRICULTURAL CHEMICALS TECHNOLOGY FOR CROP PROTECTION AND MODIFICATION

##### 1. Chemistry of Novel Plant Growth Promoting Agents (Brassins) (M. D. Grove)

- a. Specific Objective: Isolate sufficient pure brassin to complete chemical identification.

Progress: A total of 9 mg of the active growth promoter was isolated and crystallized by methods previously described. Single-crystal X-ray analysis (Naval Research Laboratory) established the structure of the growth promoter, called brassinolide, as (22R,23R,24S)-2 $\alpha$ ,3 $\alpha$ ,22,23-tetrahydroxy-24-methyl-6,7-seco-5 $\alpha$ -cholestano-6,7-lactone. Brassinolide levels as low as 10 nanograms per plant stimulate elongation, curvature, and splitting of bean second internodes.

- b. Specific Objective: Develop practical method for detection of brassin in plant material.

Progress: Preparation of model steroids is in progress.

##### Publications:

MANDAVA, N., M. KOZEMPEL, J. F. WORLEY, D. MATTHEES, J. D. WARTHEN, JR., M. JACOBSON, G. L. STEFFENS, H. KENNEY, AND M. D. GROVE. Isolation of Brassins by Extraction of Rape (Brassica napus L.) Pollen. Ind. Eng. Chem., Prod. Res. Dev. 17 (1978):351-354.

## Other Reports:

GROVE, M. D. Novel Constituents and Plant Hormonal Activity of Brassica napus (Rape) Pollen. Presented at the Society for Economic Botany meeting, St. Louis, Missouri, June 11-14, 1978.

### D. TECHNOLOGIES FOR FOOD AND FEED USES OF FIELD CROPS

#### 1. Composition and Properties of Seed Lipids for Foods and Feeds (J. A. Rothfus)

- a. Specific Objective: Complete study of thermal behavior of single acid triglycerides made from  $C_3$  to  $C_{30}$  acids.

Progress: Thermal behavior and polymorphism studies were completed on single-acid triglycerides made with saturated acids from  $C_3$  to  $C_{30}$ . Chains longer than those normal to mammalian physiology tend to impart relatively different thermodynamic properties to the saturated triglycerides. Two-carbon extensions beyond  $C_{20}$  produce  $S_f$  increases that are some fourfold smaller than those produced by similar extensions in the less than  $C_{20}$  range. Presumably this reflects inherent differences in the effect of structure on the tendency of such long chains to assume ordered conformations.

- b. Specific Objective: Develop models for whole triglycerides.

Progress: Refined procedures developed from computerized models of n-alkanes, such as polyethylene, have been applied to allow prediction of thermal properties for the more complex triglycerides. Results indicate that preferred conformations of saturated triglycerides can be assigned from calculations of associative interactions in space-filling models.

- c. Specific Objective: Restore Raman spectrophotometer to service and initiate systematic analysis of physical interactions of food and feed constituents.

Progress: Raman spectroscopy capability was restored and upgraded by acquisition and installation of a 4-watt argon ion laser and a tunable dye laser. Apparatus was designed and constructed for 180-degree sample viewing in an existing spectrometer. Initial spectra have defined sample properties and handling procedures for solvents, soybean and corn oil, sucrose and starch, albumin and casein. Analysis of organic sulfides is providing spectra of potential value to studies on sulfur in proteins. Significant differences are apparent in comparison spectra for solid and molten erucic acid.



## Publications:

EISSLER, R. L. AND J. W. HAGEMANN. Thermodynamic Properties of Fatty Acids. In Fatty Acids, edited by E. H. Pryde, American Oil Chemists' Society, Champaign, Illinois. In press.

## Other Reports:

CHANG, S. P. Polymorphism of Mono- and Diunsaturated Eicosanoic and Docosanoic Acids. Presented at the American Oil Chemists' Society meeting, St. Louis, Mo., May 14-18, 1978.

## 2. Analysis for Improved Soybean Quality (R. Kleiman)

- a. Specific Objective: Determine oil and protein content of soybean samples in order to develop improved varieties.

Progress: Over 12,000 samples were received from public soybean breeders throughout the United States and Canada. These samples were examined for their oil and protein content. In addition, more than 1180 soybean samples from the 1978 harvest were analyzed in cooperation with the Federal Grain Inspection Service. Work was started, in cooperation with Charles Brim, SEA-AR, North Carolina State University, on the analysis of 3000 soybean plant samples for nitrogen content.

- b. Specific Objective: Provide fatty acid composition of selected soybean samples in order to lower the linolenic acid content by plant breeding.

Progress: Over 700 soybean samples of the southern germ-plasm collection were analyzed by gas chromatography for fatty acid composition. In general, most samples had the usual linolenic acid content (5-10%). However, a few samples were found with linolenic acid content as low as 3.9%. In a cooperative effort with Karl Norris, SEA-AR, Beltsville Agricultural Research Center, fatty acid compositions were determined on 30 additional samples for a study on feasibility of using reflectance infrared for fatty acid determination.

- c. Specific Objective: Develop procedures for rapid analysis of soybean protein in order to raise, by breeding, the methionine and cysteine content.

Progress: A rapid procedure for methionine analysis, involving reaction of methionine in soybeans with cyanogen bromide to produce methyl thiocyanate for analysis by gas chromatography, was evaluated. The results for this amino acid are consistently 40% lower than the standard oxidation procedure, and further study is necessary.

- d. Specific Objective: Develop and improve methods for chemical analysis of soybeans.

Progress: A rapid Kjeldahl nitrogen procedure was developed which can be used on samples unsuitable for the standard reflectance

infrared method. A block digester and air scrubber were designed and constructed specifically for this method. The procedure, utilizing a single-channel Technicon autoanalyzer, was used for soybeans and soybean plant parts. The reflectance infrared procedure has also been improved. The number of samples ground per unit time has been doubled by placing six grinders in a wrist shaker and controlling the grinding time automatically. The reflectance infrared analyzer has been interfaced to a micro-computer. The computer prompts the technician, compares the data, and transmits valid data to the larger laboratory computer for permanent storage and report generation.

#### Reports:

The Uniform Soybean Tests, Northern States 1977. USDA, ARS and State Agricultural Experiment Stations, West Lafayette, IN 47907.

The Uniform Soybean Tests, Southern States 1977. USDA, ARS and State Agricultural Experiment Stations, Stoneville, MS 38776.

### E. RECLAMATION AND REVEGETATION OF LAND AREAS DISTURBED BY MAN

#### 1. Trace Element Uptake and Distribution in Agricultural Crops Grown on Disturbed Lands (K. D. Carlson)

- a. Specific Objective: Repeat experiment of growing crambe and kenaf on sludge-treated stripmine land to accumulate additional data.

Progress: Negotiations are underway to obtain a memorandum of understanding with Metropolitan Sanitary District (Chicago) to allow for planting on stripmine land in 1979.

- b. Specific Objective: Complete analyses on soil and plant samples collected in 1977. Complete analyses of data already collected.

Progress: Heavy metal analyses of crambe seed from test plots on stripmined land showed no differences due to soil amendment with sewage sludge for 3 years. Average seed analysis was: Hg, 2 ppb; Cd and Cr, 300 ppb; Pb, 600 ppb; Cu, 5 ppm; Mn, 20 ppm, Zn, 40 ppm; Fe, 75 ppm. Levels of Hg, Cd, and Pb are relatively low.

### F. NATURAL TOXICANTS AND MICROBIAL TOXINS

#### 1. Studies of Mycotoxins in Cereal Grains and Their Control (M. D. Grove)

See Fermentation Laboratory, G.1.

#### 2. Natural Toxicants in Horticultural Crops and Cruciferous Feeds (H. L. Tookey)

- a. Specific Objective: Continue survey of glucosinolate content of vegetables from the Cruciferae family.

Progress: Cambial and cortical tissues of cabbage heads contain twice the concentration of glucosinolates as do the leaves or pith. Of all glucosinolates in the leaves, those yielding thiocyanate ion comprise 68, 45, and 28% in Savoy, white, and red cabbages, respectively.

- b. Specific Objective: Correlate glucosinolate content of seeds to that of the edible vegetable.

Progress: There is no apparent correlation between glucosinolate content of seed and vegetable tissue in 14 varieties of Chinese cabbage. Butenyl glucosinolate which predominates in seed is only a minor component in the edible head.

- c. Specific Objective: Continue isolation of specific hydrolysis products from aglucons of glucosinolates for biological testing as mutagens, teratogens, or chronic toxicants.

Progress: Cyano-hydroxy-epithiobutanes show evidence of being liver and kidney toxins in rats. Two aglucon products from glucoiberin, a major glucosinolate of cabbage, have been submitted for testing as teratogens. These two plus five other aglucon products have been submitted for testing as insect oviposition factors.

- d. Specific Objective: Determine the fate of cabbage glucosinolates during fermentation to convert cabbage to sauerkraut.

Progress: All glucosinolates are hydrolyzed within the first 2 weeks of the sauerkraut fermentation. In finished kraut, thiocyanate ion was 9-17 ppm and the nitrile from glucoiberin was 16-25 ppm. No isothiocyanates or goitrin were present.

- e. Specific Objective: Isolate purified falcarinol to validate an assay method to be developed to measure this toxicant in carrots.

Progress: Falcarinol could not be isolated in sufficient amounts, apparently because of low levels in available market carrots.

- f. Specific Objective: Survey the variability of falcarinol and myristicin in market carrots.

Progress: Four varieties of carrots grown in nine geographical locations in 1978 all contained less than 1 ppm myristicin.

- g. Specific Objective: Study the specific effects of heat and moisture treatments during crambe processing to optimize processing conditions.

Progress: Thioglucosidase enzyme is 97% destroyed by heating whole seed 30 min at 82°C if the seed moisture content is 14%, but only 83% is destroyed at 9% moisture.



### Publications:

VanETTEN, C. H. Determination of Salt Content of Sauerkraut by Cation Exchange. *J. Agric. Food Chem.* 26 (1978):776.

DAXENBICHLER, M. E., C. H. VanETTEN, AND P. H. WILLIAMS. Glucosinolates and Derived Products in Cruciferous Vegetables. Analysis of 14 Varieties of Chinese Cabbage. *J. Agric. Food Chem.* 27 (1979):34-37.

VanETTEN, C. H., M. E. DAXENBICHLER, W. F. KWOLEK, AND P. H. WILLIAMS. Distribution of Glucosinolates in the Pith, Cambial-Cortex, and Leaves of the Head in Cabbage. *J. Agric. Food Chem.*, in press.

VanETTEN, C. H. Naturally Occurring Food and Feed Toxicants: Glucosinolates. In *CRC Handbook Series in Nutrition and Food*, ed., M. Rechcigl, CRC Press, West Palm Beach, Florida, in press.

VanETTEN, C. H. AND H. L. TOOKEY. Chemistry and Biological Effects of Glucosinolates. In *Interrelationship of Secondary Plant Constituents with Herbivores*, ed., G. A. Rosenthal, Academic Press, New York, in press.

MUSTAKAS, G. C. AND K. D. CARLSON. Simple Model for Determining Economic Feasibility of Processing New Oilseeds. *J. Am. Oil Chem. Soc.* 56 (1979):29-32.

### Other Reports:

VanETTEN, C. H. Chemistry and Biological Effects of the Major Glucosinolate in *Crambe abyssinica*, A Potential Oilseed Crop. Presented at the 5th International Rapeseed Conference, Malmö, Sweden, June 12-16, 1978.

SPENCER, G. F. AND M. E. DAXENBICHLER. Gas Chromatography-Mass Spectrometry of Nitriles, Isothiocyanates and Oxazolidinethiones Derived from Cruciferous Glucosinolates. Presented at the 176th National Meeting, American Chemical Society, Miami Beach, Florida, September 11-17, 1978.

MUSTAKAS, G. C. AND K. D. CARLSON. Simple Model for Determining Economic Feasibility of Processing New Oilseeds. Presented at the American Oil Chemists' Society Meeting, St. Louis, Missouri, May 14-18, 1978.

## OILSEED CROPS LABORATORY

H. J. Dutton, Chief

Research Leaders: E. A. Emken, E. N. Frankel, T. L. Mounts,  
E. H. Pryde, and W. J. Wolf

### A. PHYSIOLOGICAL AND BIOCHEMICAL TECHNOLOGY TO IMPROVE CROP PRODUCTION

#### 1. Increased Photosynthetic Efficiency of Plants Through Yellow Chloroplast Pigments (H. J. Dutton)

- a. Specific Objective: Measure action spectra of isolated soybean cells from yellow isolines of Harosoy and Clark.

Progress: Development of spectrophotometer-spectrofluorometer instrumentation for measuring action spectra of photosynthesis for various colored algae and for soybean cells from yellow mutants has taken account of new techniques, the advances and the limitations in "laser-state-of-the art." Thus optical light pipes made from high transmission fiber optics have been found to be superior in transmitting and mixing laser light to classical optical-bench procedures of lens and mirrors. Analog channels to the on-line computer have recently become available for monitoring  $O_2$  evolution, light incident, light transmitted, and light fluoresced. These channels are now programmed for data acquisition and processing. Unfortunately, the cressy violet perchlorate used in the dye laser to generate red light for Photosystem I support, while effective for short times, is so unstable and costly as to be impractical for the hour-long continuous-radiation experiments; a less intense source involving a band pass filter and a Xenon lamp has been substituted. The total system is ready to measure quantum efficiencies for oxygen evolution and for fluorescence at wavelengths primarily absorbed by carotenoids and chlorophylls of Photosystem II in order to determine which of the carotenoids of higher and lower plants serve as photosensitizers of photosynthesis.

- b. Specific Objective: Apply mass spectrometric system for determining oxygen isotopes and carbon isotopes of carbon dioxide to the study of photorespiration in cooperation with Department scientists at Urbana, Illinois.

Progress: The Bendix Time-of-Flight mass spectrometer is now able to monitor isotopes of molecular oxygen ( $^{18}O_2$  and  $^{16}O_2$ ) and carbon dioxide ( $^{13}CO_2$  and  $^{12}CO_2$ ) accurately. Changing values for mass peaks due to instrumental errors can be corrected by using an inert gas as an internal standard thereby improving precision. By placing the system on line to our central computer the data are now acquired, corrected, time averaged and plotted; and first derivatives are calculated.

Preliminary rates of photorespiration, photosynthesis, and respiration have been measured for Chlorella pyrenoidosa and compared to the literature values to confirm that work and the efficacy of our system.

Oxygen and carbon dioxide concentration can be sampled 20 or even 50 times per minute, thus allowing us to observe the photoreduction of  $O_2$  and thus the "priming" and replacement of  $CO_2$  assimilation during the first 30 seconds of a light period.

Studies of photorespiration are being conducted with whole soybean cells that are isolated using techniques developed by Department scientists at Urbana, Illinois.

- c. Specific Objective: Isolate and characterize caroteno-chlorophyll protein complexes from Phaeodactylum tricornatum which retain energy transfer capacity and make model systems of carotenoids and chlorophyll pigments exhibiting energy transfer.

Progress: Bronwill-disintegration (an abrasion process involving 0.1 mm diameter glass beads vibrating at a frequency of 30 K<sub>Hz</sub>) of the diatom Phaeodactylum tricornatum at 0-5° disrupted cells and chloroplasts to liberate carotenoid-chlorophyll a protein complexes that retained energy transfer abilities. The complex contains most of the initial carotenoid and chlorophyll a contents present in the cells but only about one-third the chlorophyll a content. Apparently, two-thirds of the chlorophyll a content is present in the residual photosystems. Unlike carotenoid-chlorophyll a protein complexes of dinoflagellates which contain both carotenoid and chlorophyll a within the same protein, complexes of diatoms are believed to exist in vivo as high molecular weight aggregates consisting of tightly packed individual chlorophyll a and carotenoid proteins. Disruption of these aggregates into smaller ones or into mixtures of individual pigmented proteins results in loss of energy transfer activity.

Model systems have been prepared by adsorbing photosynthetic pigments on surfaces of sucrose, glass, silica gel, or organosilane bonded to porous silica support. Techniques have been developed for determining fluorescence and absorption or reflectance spectra of chlorophylls and carotenoid pigments in these systems. At room temperature spectra of adsorbed chlorophyll a were similar to those of the material in solution. On hydrocarbon or organosilane surfaces fluorescence of chlorophyll a appeared to be much more intense than that of this same molecule adsorbed on powdered sugar.

- d. Specific Objective: Study the gating of electron flow between Photosystems I and II.

Progress: A polarographic method was developed to detect the production of superoxide ( $O_2^-$ ), which is sensitive to the flow of electrons between the oxygen evolving reaction center (Photosystem II) and the carbon dioxide fixing reaction center (Photosystem I) of green plants. The flow of electrons was found to be maximal after every second



flash, indicating charge flow is gated with electrons flowing two at a time; that is, electrons produced during a flash are stored and only flow when two electrons per reaction center have accumulated. This cycle of two oscillation in charge flow seems universal and was found to occur in chloroplasts of beans, dwarf peas, romaine lettuce, and spinach.

- e. Specific Objective: To simulate with a computer model the observed transition for oxygen yield per flash from the classical "cycle-of-four" pattern at high light intensity to an "S" shaped curve observed at low intensity.

Progress: A study of the changes in the oxygen flash-yield pattern at various flash intensities was carried out on algae and chloroplasts. A mathematical model to describe the behavior of oxygen-evolving systems at various flash intensities was developed based on the binomial distribution. Experimental data and calculations matched well over two orders of magnitude change in flash intensity. It was concluded from the data that there was inhomogeneity in the size of pigment aggregates associated with oxygen evolving reaction centers. Also, using a 3  $\mu$ s flash, the misses and double hits occurring in oxygen evolution were intrinsic and not photochemical in nature.

- f. Specific Objective: Expand the comparison of pigments in four genetic lines of soybeans (Harosoy and Clark) under laboratory and normal field growing conditions and under enhanced UV-B irradiation.

Progress: The study of plant pigments and their functions in growth and photosynthesis proceeded in two directions: (i) improvement of analytical techniques and (ii) theoretical relationship of pigment ratios. High-performance liquid chromatographic (HPLC) methods for chloroplast pigments were developed and sample preparation for carotenoids and chlorophylls were considerably improved. A rapid procedure of pigment sample preparation has been devised using commercially available cartridges of reverse-phase packings and millipore filters which reduces time required from 3 hours to 20 minutes. In addition new HPLC analytical techniques for flavonoids were explored as well as methods for isolation of whole cells from soybeans. Analytical techniques allowed us to relate chlorophyll deficient mutant soybean and peanut plants to etiolated and partially greened plants. Relationships were found between accessory pigments and chlorophyll a in mutants which suggests the progression of greening in the normal plant.

#### Publications:

ESKINS, KENNETH, CHARLES R. SCHOLFIELD, AND HERBERT J. DUTTON. High-Performance Liquid Chromatography of Plant Pigments. *J. Chromatogr.* 135 (1977):217-220.

ESKINS, K. AND P. J. BANKS. The Relationship of Accessory Pigments Chlorophyll a Content in Chlorophyll Deficient Peanut and Soybean Varieties. *Photochem. Photobiol.* In press.

ESKINS, K. AND H. J. DUTTON. A Rapid Method of Sample Preparation for High Pressure Liquid Chromatography of Higher Plant Pigments. Anal. Chem. In press.

ESKINS, K., F. L. THOMAS, AND H. J. DUTTON. Interaction of Chloroplast Pigment Ratios. Plant Physiol. 61 (1978):104.

GOVINDJEE AND P. JURINIC. Fast Emission Changes as Probes of Photosynthesis. Photochem. Photobiol. Rev., Vol. 4, in press.

JURINIC, P. Flash Polarographic Detection of Superoxide Production as a Means of Monitoring Electron Flow Between Photosystems I and II. FEBS Lett. 90 (1978):15-20.

JURINIC, P. Flash-Yield-Pattern for Photosynthetic Oxygen Evolution in Chloroplasts and *Chlorella* as a Function of Flash Intensity. Submitted to Arch. Biochem. Biophys.

WONG, D., GOVINDJEE, AND P. JURINIC. Analysis of Microsecond Fluorescence Yield and Delayed Light Emission Changes after a Single Flash in Pea Chloroplasts: Effects of Mono- and Divalent Cations. Photochem. Photobiol. 28 (1978):961-974.

#### Other Reports:

JURINIC, P. Flash Polarographic Detection of Superoxide Production as a Means of Monitoring Electron Flow Between Photosystem I and II. Presented at the 6th Annual Meeting of the American Society for Photobiology, June 1978, Burlington, Virginia.

### B. TECHNOLOGIES FOR FOOD AND FEED USES OF FIELD CROPS

#### 1. Effects of Isoelectric Precipitation and of Heat on Soybean Proteins (W. J. Wolf)

- a. Specific Objective: Characterize the mono-, di-, and triglycerides obtained by silica gel chromatography of the neutral lipid fraction obtained from soy protein isolate to assess their possible role as precursors of fatty acid oxidation products contributing to the flavor of soy protein isolates.

Progress: Residual lipids in protein isolates affect functional properties of the proteins and have now been evaluated as precursors of fatty acid oxidation products that may impart undesirable flavors to the isolates. Mono-, di-, and triglyceride fractions were separated from the neutral lipid fraction and tested as substrates for lipooxygenase. Although all of the glycerides were oxidized, their rate of oxidation was low; oxygen consumption was only 2-5% of that obtained with linoleic acid. Extraction of a commercial isolate and chromatography of the neutral lipid fraction also yielded mono-, di-, and

triglycerides as previously obtained from laboratory preparations of isolates. However, the ratios of the various glycerides differed from those separated from laboratory-prepared isolates.

- b. Specific Objective: Characterize the nonprotein constituents of soy protein acid-sensitive fraction (ASF) to gain fundamental knowledge regarding how these constituents contribute to the grassy/beany and bitter flavors of soybean proteins.

Progress: Although grassy/beany and bitter flavors can be dialyzed from ASF-free soy protein more rigorous procedures are necessary for removal of flavor compounds from ASF. Overnight incubation in 5% sodium dodecyl sulfate (SDS) at 50 C produced maximum dissociation of noncovalently bound nonprotein compounds from the ASF protein. Gel filtration on Sephadex G-25 fractionated the SDS-dissociated material into protein and nonprotein constituents and the amount of nonprotein material that was eluted correlated with the sample ASF content. Means are now provided for characterization of the ASF nonprotein constituents.

- c. Specific Objective: Develop a macromolecular map of soy proteins to provide a physicochemical basis for study of soy protein functionality and food system interactions.

Progress: Soy proteins were electrophoresed in analytical gels containing 0.5% agarose and polyacrylamide (0-5% T) crosslinked with N,N'-diallyltartardiamide (15% C). For the first time selective destacking of 2 components of the soy protein acid-sensitive fraction (ASF) was achieved in the separation gel; ASF is associated with soy protein grassy/beany and bitter flavors. This result provides a rationale for ASF purification and characterization. All other soy proteins remained stacked suggesting that the same electrophoretic conditions be used for steady state stacking in preparative gel electrophoresis; this would be the initial step in the development of a soy protein map.

- d. Specific Objective: Continue evaluation of fluorescence technique and initiate work on amino-terminal analysis as a possible alternate method for estimating soy in meat-soy blends.

Progress: A fluorometric technique for the quantitative determination of soy flour in meat-soy blends has been evaluated. With this rapid method, it is possible to measure texturized soy flour in meat-soy blends at the 30% level to within  $\pm 2.4\%$ . Currently a collaborative study is being conducted to examine the procedure further. Exploratory studies have been initiated on the measurement of N-terminal amino acids as a potential procedure for determining soy products in meat-soy blends.

- e. Specific Objective: Determine the feasibility and optimum conditions of the ultrasonification process for peptizing soy proteins to increase the yield of soy protein isolates above that now obtained commercially.



Progress: A continuous ultrasonification cell was set up and operated to test the peptization step by ultrasonic means as an alternate to conventional mixers. Combinations of flake-to-solvent ratio with and without ultrasonic energy were tested in replicate experiments. Although the protein was dispersed more rapidly with the sonifier in the system, overall yields of soybean isolate were not increased over that obtainable without a sonifier and using conventional dispersing systems. Additional work is needed to improve the sonification system, evaluate the protein quality, and compare cost and energy factors over that of conventional mixers.

Publications:

ELDRIDGE, A. C., L. T. BLACK, AND W. J. WOLF. Carbohydrate Composition of Soybean Flours, Protein Concentrates, and Isolates. J. Agric. Food Chem. In press.

ELDRIDGE, A. C. AND L. G. HOLMES. Evaluation of a Fluorometric Technique for Quantitative Determination of Soy Flour in Meat-Soy Blends. J. Food Sci. In press.

RHA, C. K., J. FLINK, N. CATSIMPOOLAS, M. KAREL, A. SINSKEY, S. TANNENBAUM, D. I. C. WANG, D. HSIEH, S. J. CIRCLE, W. E. HARTMAN, W. H. MARTINEZ, H. SCHOEN, AND W. J. WOLF. Basic Food Science and Technology Problems Affecting the Properties and Processing of Protein Resources. In: Protein Resources and Technology, edited by M. Milner, N. S. Scrimshaw, and D. I. C. Wang, AVI Publishing Co., 1978.

WOLF, W. J. New Soy Protein Food Products in the United States. Proceedings International Soya Protein Food Conference, Singapore, pp. 59-65, 1978.

WOLF, W. J. Soybean Proteins: Their Production, Properties, and Food Uses. A Selected Bibliography. Proceedings International Soya Protein Food Conference, Singapore, pp. 121-126, 1978.

Other Reports:

ELDRIDGE, A. C. Determination of Soy Products in Meat-Soy Blends. Presented at Soybean Research Council, National Soybean Processors Association, Peoria, Illinois, April 1978.

ELDRIDGE, A. C. Determination of Soy Products in Meat-Soy Blends. Presented at Food Protein Council, Scientific Committee Meeting, Peoria, Illinois, September 1978.

MOULTON, K. Continuous Ultrasonic Extraction of Soy Protein to Produce ISP. Presented at Food Protein Council Scientific Committee Meeting, NRRC, Peoria, Illinois, September 1978.

WOLF, W. J. Soy Protein Processing for Human Food. Presented at International Soya Protein Food Conference, Singapore, Republic of

Singapore, January 1978 and at a meeting of Food Development (Shokuhin Kaihatsu), Tokyo, Japan, February 1978.

WOLF, W. J. New Soy Protein Food Products in the United States. Presented at International Soya Protein Food Conference, Singapore, Republic of Singapore, January 1978 and at a meeting of Food Development (Shokuhin Kaihatsu), Tokyo, Japan, February 1978.

WOLF, W. J. Soybean Protein Research at Northern Regional Research Center. Presented at National Food Research Institute, Tokyo, Japan, January 1978.

WOLF, W. J. Soybean Ultrastructure, and Physical and Chemical Properties of the Proteins. Presented at seminar, Quaker Oats Company, Barrington, Illinois, May 1978.

WOLF, W. J. Processing Factors Affecting Quality of Edible Soy Flours; Edible Soybean Proteins and Their Uses; and Flavor, Flatus Factors and Nutritional Properties of Soy Flours. Presented at seminars to Department of Technology, Ministry of Industry, Commerce, and Tourism, La Paz, Bolivia, September 1978.

WOLF, W. J. Nomenclature of Soy Protein Flavor, AACC Flavor Subcommittee Activities. Presented at Food Protein Council, Scientific Committee Meeting, Peoria, Illinois, September 1978.

WOLF, W. J. Long-term Feeding Studies on Effects of Residual Trypsin Inhibitor Activity in Commercial Soybean Proteins. Presented at Collaborators' Conference, Peoria, Illinois, October 1978.

2. cis-Bond Forming Hydrogenation and Autoxidation Studies to Improve Edible Soybean Oil (E. N. Frankel)

- a. Specific Objective: Study thermal and homolytic decomposition of autoxidation products to determine volatile compounds causing offensive flavors and odors and the interaction of such products with soybean proteins and amino acids and other biological effects.

Progress: Purified hydroperoxides from methyl oleate, linoleate, and linolenate were thermally decomposed to determine their importance as precursors of offensive flavors and odors in autoxidized vegetable oils. The major volatile compounds identified by gas chromatography-mass-spectrometry (GC-MS) from oleate 8-, 9-, 10-, 11-hydroperoxides included C<sub>7</sub>- and C<sub>8</sub>-hydrocarbons, C<sub>8</sub>- and C<sub>9</sub>-saturated aldehydes, C<sub>9</sub>- and C<sub>10</sub>-unsaturated aldehydes, C<sub>7</sub>-, C<sub>8</sub>-, and C<sub>9</sub>-methyl esters. These volatile compounds are the same as those previously identified in triolein heated in air at 192 C (see B.3.). Therefore, hydroperoxidation is an important process occurring during frying with oils. The volatile products from linoleate 9- and 13-hydroperoxides included pentane, hexanal, 2,4-decadienal, methyl azelaaldehyde and 13-oxo-trideca-9,11-dienoate, a new and previously unreported decomposition product. Methyl azelaaldehyde, a major decomposition product of



oleate, linoleate, and linolenate hydroperoxides, proved to be reactive with various soybean proteins. (See B.7.)

The pathways of homolytic degradation of linoleic hydroperoxides have now been largely defined. Isomers of linoleic acid hydroperoxide degraded homolytically to form stereo- and regio-specific fatty epoxides. This observation established that epoxides are formed exclusively by intramolecular ring closure involving the hydroperoxy group. Two of these epoxides, either 9-hydroxy-trans-12,13-epoxy-trans-10-octadecenoic acid or 13-hydroxy-trans-9,11-epoxy-trans-11-octadecenoic acid, served as precursors in the formation of a mixture of the delta-ketols, 9(13)-oxo-13(9)-hydroxy-trans-11(10)-octadecenoic acids. Additionally, the above epoxides easily solvolyzed to trihydroxy fatty acids. 9-Oxo-trans-12,13-epoxy-trans-10-octadecenoic acid was demonstrated to react with glycine to form glycine-fatty acid adducts. The enzyme, linoleic acid hydroperoxide isomerase, was shown to act through an  $SN_2$  mechanism; that is, a nucleophile substituted at the hydroperoxy group inverting the optical center. In cooperation with SEA-AR's Western Regional Research Center, a number of fatty acid oxidation products were isolated for mutagenicity tests by the Ames Method.

In collaboration with Professor P. Budowski (Project 1002: US-Israel Binational Science Foundation) a polar lipid extract from oxidized safflower oil esters accelerated the induction of encephalomalacia in young chicks as did the ketoene and keto-diene fatty acids. The possibility is suggested that ketopolyenoic fatty acids from oxidized oils react with amino lipids or proteins in causing damage to cell membranes.

- b. Specific Objective: Prepare and evaluate heterogeneous and homogeneous catalysts for nutritionally desirable cis-bond forming hydrogenation.

Progress: Soluble linear polydiphenylsiloxane-chromium carbonyl and high molecular weight "ladder" polyphenylsilsequioxane-chromium carbonyl were prepared as cis-producing hydrogenation catalysts. These siloxane polymer catalysts were more thermally stable than the corresponding polystyrene complexes and were active at 200 C or above. However, these catalysts lost chromium carbonyl during hydrogenation. An insoluble polymer bound triphenylphosphine-chromium carbonyl complex showed good catalytic activity but was not more thermally stable than polystyrene-chromium carbonyl.

In acetone at 140 C, crosslinked polystyrene chromium carbonyl catalyst could be recycled four times. However, too much chromium was lost on recycling causing loss of activity. In cyclohexane at 200 C, the linolenate content of soybean oil was reduced to 1.3% in 6 hours, the I.V. to 100 and the trans content of the products to less than 3%. Dissociation of chromium carbonyl from the polymer during hydrogenation was shown by loss of chromium from the polystyrene chromium carbonyl catalyst and by the presence of soluble chromium in the hydrogenated product.



Soybean esters were selectively conjugated with potassium tertiary butoxide in tetraglyme. Methyl linolenate was thus conjugated seven times faster than methyl linoleate. However, this procedure produced minor amounts of tertiary butyl esters and did not work with triglycerides. When the conjugated soybean esters were hydrogenated with different homogeneous and heterogeneous chromium carbonyl catalysts the linolenate-to-linoleate selectivity varied from 7.2 to 10.5 compared to 1.8 for the nonconjugated esters.

- c. Specific Objective: Study the mechanism of antioxidant action of tocopherols in soybean oil and the effect of other additives to determine how to improve flavor stability.

Progress: Diminished rates of oxidation resulted from the addition of alpha-tocopherol and beta-carotene to soybean oil methyl esters, and a "normal" isomeric hydroperoxide distribution was found as expected from the fatty acid composition of the starting material. This result is in contrast to previous analyses of soybean esters showing unexpectedly high amounts of 12-hydroperoxide at low peroxide values. The evidence that this unique hydroperoxide arises from linoleate oxidation by singlet oxygen is supported by results reported under new objective d. Both alpha-tocopherol and beta-carotene apparently act by quenching the formation of singlet oxygen.

- d. Specific Objective: Study the effect of singlet oxygen on oxidative deterioration of vegetable oils.

Progress: Different vegetable oil esters were autoxidized under the same conditions as soybean oil esters to compare the isomeric hydroperoxide composition at different peroxide values. As in soybean oil esters, cottonseed and safflower oil esters, free of linolenate, also showed the presence of a unique 12-hydroperoxide, when oxidized at low levels. Several lines of evidence support the conclusion that singlet oxygen may contribute to the unique hydroperoxide composition of vegetable oil esters oxidized at low peroxide values. Methylene blue and chlorophyll increased the level of 10-, 12-, and 15-hydroperoxides. These products are those expected from oxidation with singlet oxygen. Treating soybean oil with carbon-black to remove natural photosensitizers produced a normal hydroperoxide distribution. GC-MS analysis of weakly oxidized samples of soybean esters (after  $\text{NaBH}_4$  reduction) provided indirect evidence for a 12-hydroxy-9,13-diene expected by photosensitized oxidation of linoleate. Traces of photosensitizers in soybean esters apparently catalyze the oxidation of linoleate by singlet oxygen at low peroxide values.

#### Publications:

AWL, R. A., E. N. FRANKEL, J. P. FRIEDRICH, AND E. H. PRYDE. cis-Bond-Producing Hydrogenation of Polyunsaturates Catalyzed by Polymer Complexed  $\text{Cr}(\text{CO})_3$  Catalysts. J. Am. Oil Chem. Soc. 55 (1978):577.

FRANKEL, E. N., R. A. AWL, AND J. P. FRIEDRICH. cis-Unsaturated Fatty Acid Products by Hydrogenation with Chromium Carbonyl Catalysts. In Catalysis in Organic Synthesis, Edited by W. H. Jones, Academic Press (in press).

FRANKEL, E. N. AND W. E. NEFF. Analysis of Autoxidized Fats by GC-MS. IV. Soybean Oil Methyl Esters. Lipids 14 (1979):39-46.

FRANKEL, E. N. Autoxidation. In Fatty Acids, Edited by E. H. Pryde, American Oil Chemists' Society, Champaign, Illinois, Chapter 18 (in press).

GARDNER, H. W. Stereospecificity of Linoleic Acid Hydroperoxide Isomerase from Corn Germ. Lipids (in press).

GARDNER, H. W. Lipid Hydroperoxide Reactivity with Proteins and Amino Acids: A Review. J. Agric. Food Chem. (in press).

NEFF, W. E., E. N. FRANKEL, C. R. SCHOLFIELD, AND D. WEISLEDER. High-Pressure Liquid Chromatography of Autoxidized Lipids. I. Methyl Oleate and Linoleate. Lipids 13 (1978):415.

#### Other Reports:

AWL, R. A. cis-Bond-Producing Hydrogenation of Polyunsaturates Catalyzed by Polymer Complexed  $\text{Cr}(\text{CO})_3$  Catalysts. Presented at American Oil Chemists' Society Meeting, St. Louis, Mo., May 14-18, 1978.

FRANKEL, E. N. Analysis of Autoxidized Fats by Gas Chromatography-Mass Spectrometry. Soybean Oil Methyl Esters. Presented at American Oil Chemists' Society meeting, St. Louis, Mo., May 14-18, 1978.

FRANKEL, E. N. High-Pressure Liquid Chromatography of Autoxidized Lipids. Methyl Oleate and Linoleate. Presented at American Oil Chemists' Society Meeting, St. Louis, Mo., May 14-18, 1978.

FRANKEL, E. N. cis-Unsaturated Fatty Acid Products by Hydrogenation with Chromium Carbonyl Catalysts. Presented at the Seventh Conference on Catalysis in Organic Syntheses, The Organic Reactions Catalysis Society, Chicago, Illinois, June 5-7, 1978.

FRANKEL, E. N. Stereochemical Synthesis of Dienol Isomers for Mechanistic Studies of Linoleate Autoxidation. Presented at 14th World Congress at the International Society for Fat Research, Brighton, England, September 17-22, 1978.

GARDNER, H. W. Stereospecificity of Linoleic Acid Hydroperoxide Isomerase from Corn Germ. Invited symposium on "Lipoxygenase," ACS Meeting, Anaheim, California, March 12-17, 1978.



GARDNER, H. W. Lipid Hydroperoxide Reactivity with Proteins and Amino Acids: A Review. Invited symposium on 'Metabolism of Polyunsaturated Fatty Acids,' Presented at American Oil Chemists' Society Meeting, St. Louis, Mo., May 14-18, 1978.

GARDNER, H. W. Further Studies of a Model for Lipid Hydroperoxide Degradation: Stereo- and Regio-selectivity of the Decomposition. Presented at 14th World Congress at the International Society for Fat Research, Brighton, England, September 17-22, 1978.

GARDNER, H. W. Highlights of Recent Research at the Northern Regional Research Center, invited seminar at Lord Rank Research Centre, England, September 15, 1978.

3. Mass Spectrometry of Deuterium Labeled Blood Lipids, Soybean Oil Odors, and Other Research Samples (W. K. Rohwedder)

- a. Specific Objective: Increase accuracy and sensitivity of mass spectral analysis of human blood lipid samples.

Progress: Over 500 deuterium triple labeled blood lipid samples from the study of metabolism of fats in humans (see E.1.) were analyzed by gas chromatography multiple ion monitoring mass spectrometry. The new Finnigan gas chromatography-mass spectrometry system has been evaluated and with the computer programming provides wide dynamic range and correction for mass defect. Computer programs for selected ion monitoring have been written but not extensively tested. Preliminary results from chemical ionization mass spectrometry application to fatty esters indicate an increase in instrument sensitivity can be achieved by this technique.

- b. Specific Objective: Identify compounds causing offensive room odors during deep fat frying which limit sales of soybean oil in Europe.

Progress: The study of volatiles from trilinolein has been completed. A compound not previously reported in volatiles from oils was identified. This compound, 4,5-epoxydec-2-enol, was found at the 3% level and identified by comparing our spectra with those obtained from P. A. T. Swoboda of the Research Council, Norwich, England. Compounds formed during thermal decomposition of methyl oleate hydroperoxide were identified by gas chromatography-mass spectrometry in cooperation with project B.2. using knowledge and methods developed for heated soybean oil and room odor analysis. This work showed that the decomposition products from hydroperoxides are similar to the volatiles from heated soybean oil. Identification of heated oil volatiles and hydroperoxide decomposition products provides basic information needed to increase the marketability of soybean oil by eliminating objectionable odors.

- c. Specific Objective: Collaborate with other NRRC scientists in mass spectrometric structural analysis.



Progress: The magnetic deflection and quadrupole mass spectrometers have been used to study a wide range of problems. Almost 500 hydroxy esters were run as TMS derivatives in conjunction with project B.2. in the determination of the positions of hydroperoxides for mechanism studies. The high resolution mass spectrometer was used to measure the molecular weight of 36 samples to an accuracy of one millimass; from these data atomic compositions could be specified for use in structure determination and in place of a derivative formation and other classical methods of calculating empirical formulas. These samples included mycotoxins, materials extracted from natural products, polysulfide compounds, synthetic intermediates and final products. The chemical ionization mass spectrometer was used to generate molecular ions from samples which do not normally give molecular ions including two intermediates from a synthesis of a growth stimulant and a suspected toxin. The quadrupole mass spectrometer has been used in a survey of moldy corn to look for vomitoxin, T-2 toxin and diacetoxysciperol. The instrumentation was also used to determine deuterium in synthesized products and nitrogen-15:nitrogen-14 ratios from nitrogen fixation experiments in algae.

Publications:

SELKE, E., E. N. FRANKEL, AND W. E. NEFF. Thermal Decomposition of Methyl Oleate Hydroperoxides and Identification of Volatile Components by Gas Chromatography-Mass Spectrometry. *Lipids* 13(7) (1978):511-513.

ROHWEDDER, W. K., W. F. KWOLEK, D. J. WOLF, AND W. L. EVERHART. Mass Spectrometric Analysis of Deuterium Dual Labeled Blood Lipids. Accepted Biomedical Mass Spectrometry. 1979.

BUTTERFIELD, R. O., W. K. ROHWEDDER, E. D. BITNER, J. O. ERNST, D. J. WOLF, AND H. J. DUTTON. Computers in the Lipid Research Laboratory. *Progress in Lipid Research* 17(1) (1978):93-110.

Other Reports:

SELKE, E. Identification of Volatiles in Heated Triglycerides. Presented at Conference of General Collaborators, NRRC, Peoria, Illinois, October 23-24, 1978.

SELKE, E. Thermal Decomposition of Methyl Oleate Hydroperoxides and Identification of Volatile Components by Gas Chromatography-Mass Spectrometry. Presented at American Oil Chemists' Society Meeting, St. Louis, Mo., May 14-18, 1978.

SELKE, E. Volatile Components from Trilinolein Heated in Air. Presented at American Oil Chemists' Society Meeting, St. Louis, Mo., May 14-18, 1978.

4. Continuous Hydrogenation of Soybean Oil for Export and Domestic Markets  
(T. L. Mounts)

- a. Specific Objective: Investigate continuous hydrogenation in laboratory-scale, high pressure apparatus to acquire data to simulate the kinetics and understand the mechanism of copper catalyzed hydrogenation of soybean oil under these reaction conditions.

Progress: A series of reactions were performed under an experimental design involving three independent variables--temperature (155-225 C), pressure (75-210 psi), and concentration of copper catalyst (0.15-1.85%). Oil flow was 100 ml/min. For all reactions (20), compositions of final and intermediate samples were determined. Conjugated diene content decreased with increasing pressure and was less than 1.0% in all samples from runs at 140 and 210 psi. trans Formation increased in a straight line with decrease in iodine value. Selectivity remained high in all reactions. The results contribute to the understanding of the continuous hydrogenation process and the kinetics and mechanisms of catalysts by copper. This information is needed for the hydrogenation of soybean oil to a stable cooking oil.

- b. Specific Objective: Finalize specifications for purchase of continuous high pressure reaction system, acquire system, utilize system to perform continuous high pressure hydrogenations.

Progress: Specifications for purchase of continuous high pressure reaction system were completed, bids were advertised and system was ordered. All components for the system have been received except for the fabricated reactor. Delivery is expected during March 1979.

- c. Specific Objective: Conclude investigation of the effect of high pressure on the mechanism of the copper catalyzed reduction of fatty acids.

Progress: Investigations of the effect of high pressure in dead-end batch reactions on the mechanism of copper-catalyzed reduction of fatty acid esters were completed. Observations were (1) at 1000 psi, 95% of the double bonds in linoleate conjugated prior to hydrogenation, conjugated double bonds reacted much faster than methylene interrupted double bonds, and linolenate selectivities were ca 9, (2) at 15,000 and 30,000 psi, selectivity decreased to below 6, probably because a part of the polyunsaturated fatty acids is hydrogenated directly without prior conjugation, and (3) at pressures ranging from 50 to 500 psi, the rate of hydrogenation was directly proportional to pressure and conjugated dienes were completely absent above 200 psi. These observations contribute to the basic knowledge of copper catalyzed hydrogenation which is needed to produce stable cooking oil from soybean oil.

- d. Specific Objective: Evaluate aluminum alkyls as catalysts in batch and continuous hydrogenation of soybean oils for improving nutritional quality of consumer products.

Progress: Aluminum alkyl-promoted nickel catalysts were used to hydrogenate soybean oil that was degummed only, and soybean oil that



was degummed, refined, and bleached. Preliminary experiments indicate that Ziegler type trialkyl aluminum-nickel catalysts are extremely active at low temperatures. However, linoleate selectivity was poor at 125 C. Catalyst activity was the same regardless of the lot of oil used. This indicates that the catalyst is different from commercial catalysts, which are easily poisoned with degummed soybean oil.

- e. Specific Objective: Complete construction of room odor facilities, standardize procedure for room odor evaluations, train room odor panel, and evaluate edible oils for room odor characteristics for work done on maintenance and expansion of foreign markets.

Progress: Construction of room odor facilities was completed. Procedures for room odor evaluation were standardized. A scoring system was tested based on quantitative descriptive analysis, that uses an unstructured line anchored at the end with contrasting terms (i.e., weak and strong). The judge evaluates the intensity of each odor description by placing a vertical line across the unstructured line. Panel was trained in the use of this scoring system and a basic list of odor descriptions was formulated.

- f. Specific Objective: Extend study of the efficacy of antioxidants for stabilization of soybean oils to evaluate requirements to ensure a nutritious edible oil for United States diets.

Progress: A study to determine the efficacy of TBHQ for flavor stabilization of citrated soybean oils indicated that TBHQ was no more effective than BHA/BHT for flavor stabilization during storage and low-temperature use.

- g. Specific Objective: Continue development of techniques needed for checking edible oil quality, including separation of components and analysis of continuously hydrogenated soybean oil and other applications of high-performance liquid chromatography and capillary gas chromatography.

Progress: Preparative high performance liquid chromatography (HPLC) was investigated as an alternative to counter double current distribution (CDCD) for the separation and purification of fatty acid methyl esters. Linolenate was recovered from linseed oil methyl esters in 98.5% purity. For isolation of 10 grams or less quantities of linolenate preparative HPLC is a suitable alternative to CDCD.

HPLC with silver resin columns (44-53  $\mu$  XE 284 resin) gave good separations of cis and trans monoene and diene isomers. Conjugated diene and triene esters were eluted and were well resolved. cis,cis Positional diene isomers of the type produced during hydrogenation of soybean oil and of linolenate were well resolved. Retention volumes and indices for compounds were determined.

As an alternative to the tedious alkali-isomerization method, linolenic acid can be quantitatively estimated in the presence of conjugated



dienes by a new GLC method using a mixture of OV-17 and OV-225 as a separating column.

- h. Specific Objective: Modify the method of SEA-AR's Southern Regional Research Center for objective evaluation of oil flavor to facilitate use with existing equipment.

Progress: Volatile analysis of edible oils was performed with glass inserts in the injection port of the Varian A-3700 GC instrument. A 9-foot column of 10% poly-MPE on Tenax-GC (60/80 mesh) support was used to fractionate the volatiles. Volatiles were generated from 150 mg of oil, at 190 C and collected on the GC column held at 40 C for 40 minutes. Temperature programming to 190 C at 3°/min gave elution of the volatiles. The method was applied to the series of aged oil samples of the AOCS Flavor Nomenclature Committee collaborative study "Correlation of Objective with Subjective Methods of Flavor Score Evaluation." Correlation of results has not been published by the committee at this time.

Publications:

BITMAN, J., D. L. WOOD, AND T. L. MOUNTS. Effect of Corn Oil and Hydrogenated Corn Oil on Egg Yolk Cholesterol. *Lipids* (in press).

KORITALA, S. Quantitative GLC Determination of Methyl Linolenate in the Presence of Conjugated Dienes. *J. Am. Oil Chem. Soc.* (in press).

DUTTON, H. J. Hydrogenation of Fats and Its Significance. AOCS Monograph "Isomeric Fats," Edited by E. A. Emken and H. J. Dutton (in press).

MOUNTS, T. L., S. KORITALA, J. P. FRIEDRICH, AND H. J. DUTTON. Selective Hydrogenation of Soybean Oil. IX. Effect of Pressure on Copper Catalysis. *J. Am. Oil Chem. Soc.* 55 (1978):402-406.

MOUNTS, T. L., G. R. LIST, K. A. WARNER, S. KORITALA, AND J. P. FRIEDRICH. Flavor and Oxidative Stability of Hydrogenated and Unhydrogenated Soybean Oils. Effects of Antioxidants. *J. Am. Oil Chem. Soc.* 55 (1978):345-349.

MOUNTS, T. L. Hydrogenation. AOCS Monograph "Fatty Acids," Edited by E. H. Pryde (in press).

MOUNTS, T. L. Odor Considerations in the Use of Frying Oils. *J. Am. Oil Chem. Soc.* (in press).

SCHOLFIELD, C. R. Silver Nitrate High-Performance Liquid Chromatography of Fatty Methyl Esters. *J. Am. Oil Chem. Soc.* (in press).

SCHOLFIELD, C. R., R. O. BUTTERFIELD, AND H. J. DUTTON. Calculation of Catalyst Selectivity. *J. Am. Oil Chem. Soc.* (in press).

SNYDER, J. M., H. J. DUTTON, AND C. R. SCHOLFIELD. Laboratory-Scale Continuous Hydrogenation. J. Am. Oil Chem. Soc. 55 (1978):383-386.

SNYDER, J. M., C. R. SCHOLFIELD, AND T. L. MOUNTS. Laboratory-Scale Continuous Hydrogenation: Copper Catalysis. J. Am. Oil Chem. Soc. (in press).

Other Reports:

MOUNTS, T. L. Soybean and Soybean Oil Processing, Series of Seminars presented at ASOGRAS, Caracas, Venezuela, November 6-9, 1978.

MOUNTS, T. L. Review of Edible Oil Research at NRRC, presented at Dwight P. Joyce Research Center, Durkee Foods, Strongsville, Ohio, February 7, 1978, and at Best Foods Research and Development Center, CPC, Int., Union, New Jersey, December 1, 1978.

TALLENT, W. H. Hydrogenation and Nutritional Quality of Vegetable Oils. Presented at Society of Chemical Industry (Oils and Fats Group), London, England, December 7, 1978.

5. Upgrading Quality of Products from Damaged or Contaminated Soybeans  
(T. L. Mounts and G. R. List)

- a. Specific Objective: Evaluate effects on oil quality of storage of soybeans damaged by harvesting equipment.

Progress: Two varieties of soybeans (Williams and Beeson) were harvested at three moisture levels (11%, 13%, 17%) and three peripheral cylinder speeds (430, 600, 750 rpm). Beans were stored in on-farm type storage bins with temperature monitoring and control. Sampling was performed at 0, 3, 7, and 10 months' storage. A final sample will be taken after 22 months' storage. Sixty samples, 2 kg each, were obtained at each period. Crude oils extracted from bean samples were analyzed for peroxide value, free fatty acids, iron content and phosphorous content. A rapid method for digestion of crude oil for phosphorus determination was evaluated and applied to the determination of phosphorus in soybean oil for the first time (Morrison, W. R., Anal. Biochem. 7:218-224, 1964). Variations in harvesting conditions have shown no effect on the free fatty acid content of oil from soybeans stored for up to 10 months. Iron content of oil appeared to decline with storage.

This study was conducted in cooperation with the Agricultural Experiment Station, USDA, University of Illinois and the Department of Agricultural Engineering, University of Illinois.

- b. Specific Objective: Ascertain the heavy metal content of oil extracted from soybeans grown on sludge-treated soil and their fate during processing.

Progress: Soybeans grown on strip-mined land treated with urban sludge were obtained from the Agronomy Department, University of

Illinois. A new graphite furnace (HGA 2200) Atomic Adsorption Spectrometer, Perkin Elmer 372, having much greater sensitivity than the previous instrument, was acquired, installed, and standardized against NBS AA standards.

- c. Specific Objective: Study the effect of hydrogen peroxide on the color of fluid and plastic lecithins.

Progress: A single lot of commercially extracted crude soybean oil was degummed with water and hydrogen peroxide to produce single bleached lecithin. It was found: (1) bleaching is both time and peroxide-concentration dependent; (2) bleaching is rapid in the initial stages but levels off with time; and (3) somewhat longer degumming times are required.

- d. Specific Objective: Characterize the residual metal content of commercial edible oil products.

Progress: Presence of trace metals in commercial products has been cited as a possible health hazard. There is little published data on the levels of trace metals in commercial edible oil products. Graphite furnace combustion conditions were established for atomic adsorption determination of trace metals in commercial hydrogenated oils. Three samples, on hand, were analyzed. Levels of trace metals were in the following ranges: Nickel 0.5-0.9 ppm, iron 0.22-0.44 ppm; chromium 0.03-0.24 ppm, copper 0.13-0.18 ppm, zinc 0.01-0.05 ppm and phosphorus 5.1-22.0 ppm. A series of commercial soybean oil products (cooking oils--5, margarines--5), in duplicate, were obtained from local groceries. Base oils were recovered from margarines for analysis.

- e. Specific Objective: Complete report on the current state-of-the-art in soybean oil processing technology as specified under Cooperative Agreement No. 12-14-3001-739 with the American Soybean Association.

Progress: Because of the detailed treatment being given soybean oil processing technology and the resultant increased length of the handbook, the Cooperative Agreement was extended 6 months to March 31, 1979. Of the 22 sections listed in the original outline, 17 have been completed or are being sent through the review process. The remaining 5 sections will be completed shortly. Three commercial oil processing plants were visited by the principal investigators to confirm actual operating practices.

- f. Specific Objective: Continue to provide library and other facilities for the development of an annotated bibliography of published research reports on soybean oil processing, properties, and edible uses as specified under Cooperative Agreement No. 12-14-3001-740 with the American Soybean Association.

Progress: Abstracts of selected references based on computer printout (1970-1977) and citations in the collective indices of Chemical



Abstracts prior to 1970 have been placed on 8 X 10 cards. Keywording of these abstracts to develop a dictionary of terms is in progress.

#### Publications:

BREKKE, O. L. Edible Oil Processing--Introduction. ASA Handbook: Soybean Oil Processing Technology (in press).

BREKKE, O. L. Specifications for Soybean Oil. ASA Handbook: Soybean Oil Processing Technology (in press).

DOTY, H. O., JR. U.S. and World Soybean Oil Markets. ASA Handbook: Soybean Oil Processing Technology (in press).

LIST, G. R., C. D. EVANS, L. T. BLACK, AND T. L. MOUNTS. Removal of Iron and Phosphorus by Commercial Degumming of Soybean Oil. J. Am. Oil Chem. Soc. 55 (1978):275-276.

LIST, G. R., T. L. MOUNTS, K. WARNER, AND A. J. HEAKIN. Steam Refined Soybean Oil. I. Effect of Refining and Degumming Methods on Oil Quality. J. Am. Oil Chem. Soc. 55 (1978):277-279.

LIST, G. R., T. L. MOUNTS, AND A. J. HEAKIN. Steam Refined Soybean Oil. II. Effect of Degumming and Refining Methods on Removal of Prooxidants and Phospholipids. J. Am. Oil Chem. Soc. 55 (1978):280-284.

LIST, G. R., A. J. HEAKIN, C. D. EVANS, L. T. BLACK, AND T. L. MOUNTS. Factor for Converting Elemental Phosphorus to Acetone Insolubles in Crude Soybean Oil. J. Am. Oil Chem. Soc. 55 (1978):521-522.

LIST, G. R., G. F. SPENCER, AND W. H. HUNT. Toxic Weedseed Contaminants in Soybean Processing. J. Am. Oil Chem. Soc. (in press).

MOUNTS, T. L. Recent Developments in Vegetable Oil Processing Technology. Grasa Y Aceites (in press).

MOUNTS, T. L. Vegetable Oils. Aceite Pub. (in press).

MOUNTS, T. L. Post-Harvest Retention of Quality of Soybeans, Proceedings of the 27th Oilseed Processing Clinic (1978).

MOUNTS, T. L., G. R. LIST, AND A. J. HEAKIN. Post-Harvest Handling of Soybeans: Effect on Oil Quality. J. Am. Oil Chem. Soc. (in press).

MUSTAKAS, G. C. Recovery of Oil from Soybeans. ASA Handbook: Soybean Oil Processing Technology (in press).

WARNER, K. A., C. D. EVANS, G. R. LIST, H. P. DUPUY, J. I. WADSWORTH, AND G. E. GOHEEN. Flavor Score Correlation with Pentanal and Hexanal Contents of Vegetable Oils. J. Am. Oil Chem. Soc. 55 (1978):252-256.

6. Dietary Fibers and Residual Lipids in Defatted Soybean Protein Products  
(J. J. Rackis)

- a. Specific Objective: Complete the identification of the furaldehyde compound and establish the role of peroxidase in the coupled oxidation of soy phosphatidylcholine (SPC) with lipoyxygenase in relation to the formation of bitter taste, which limits acceptability of soy proteins in foods.

Progress: 5-(Pentenyl)-2-furaldehyde and chloromethyl furaldehyde were isolated from phospholipids of defatted soy flakes following treatment with HCl gas. The chloromethyl derivative may be an artifact. Horseradish peroxidase (HRP) and bovine blood hematin catalyzed the oxidation of liposomes of sonicated SPC. An oxygen uptake rate of 1.16  $\mu\text{moles/min/mg}$  protein was obtained with HRP catalysis, which was three times greater than that observed with soy lipoyxygenase. A very much greater oxygen uptake of 9  $\mu\text{moles/min/mg}$  sample was observed in hematin-catalyzed systems. Heat denaturated HRP increased oxygen uptake tenfold with linoleic acid substrates, but, decreased uptake 20% with polyunsaturated fatty acid moieties in SPC substrates. These observed differences in oxidation cannot be explained. Catalyzed oxidation of fatty acids and SPC with HRP, hematin and UV light generated a vast array of oxygenated fatty acids. No furaldehyde derivatives were present in these three systems. Soybean peroxidases have been isolated. However, lipoyxygenase contaminants must be removed before the role of peroxidases in catalyzing the formation of objectionable flavors can be evaluated.

- b. Specific Objective: Develop procedures to separate and quantitate the various forms of SPC to establish the relationship between extent of oxidation and their contribution to the poor flavor qualities of soy protein products.

Progress: A high pressure liquid chromatographic technique has been developed to separate and quantitate oxidized and unoxidized forms of soy phsophatidylcholine (SPC). Reverse phase chromatography in 95% methanol-water resolves oxidized SPC, as determined by absorbance at 234 nm, from the unoxidized molecular species. The ratio of oxidized to unoxidized species can be determined by quantitation of 206 nm absorbance.

Gas chromatographic analysis of newly formed oxidized species indicate that only one of the two fatty acids on the molecule is oxidized. Mass spectral analyses show that the oxidized fatty acid is an epoxide, oxo, mono- or di-hydroxide derivative. Individual molecular species of SPC have been isolated and purified.

- c. Specific Objective: Continue the studies on the digestibility of soy, corn, and wheat bran and characterize the soluble nondigested carbohydrates and protein to establish the nutritional significance of dietary fiber.

Progress: Ultrafiltration procedures were developed to isolate soluble indigestible residue (IDR) from mammalian enzyme digests of soy protein products, soy hulls, and cereal brans. Soluble IDR, which contains protein and carbohydrate material, increased total IDR by 3 to 160%. The soluble IDR carbohydrates from soy products contained high amounts of arabinose and galactose whereas that from cereal brans was high in glucose. The nonsoluble IDR of soybean meal and protein concentrate had higher proportions of hemicellulose but lower levels of cellulose and lignin compared to soybean hulls. The percent protein digestibilities values for soy protein products were: whole soybeans 68%; hulls 60%; dehulled, defatted flakes 81%; protein concentrate 61%. In cereal brans, percent protein digestibility values were: corn 43%, wheat 60%.

These data should provide more complete characterization of dietary fiber in soybeans for investigating their physical properties when eaten by man.

- d. Specific Objective: Evaluate the effect of prolonged consumption of soy protein on vitamin B<sub>12</sub> requirements of rats to establish in part the health and safety aspects in human nutrition of soy protein, which is becoming increasingly important as a world source of protein.

Progress: Significantly increased liver and kidney weights relative to body weights were noted in rats fed a soy protein concentrate (containing 310 mg trypsin inhibitor (TI)/100 g diet) in vitamin B<sub>12</sub> deficient diets; the rats also lost weight during the feeding trial of about 300 days. B<sub>12</sub> Administration brought about an immediate stimulation of growth attributable to an increase in feed consumption. No significant differences in organ weights were noted in rats fed soy flour and soy protein isolate diets containing 176 mg TI/100 g diet compared with casein-fed groups. Addition of B<sub>12</sub> promoted growth with all soy proteins but was without effect in casein diets. Microscopic examination of pancreata revealed no abnormalities in B<sub>12</sub> deficient rats. These data suggest that soy diets containing relatively low residual TI activity, when balanced with essential nutrients, do not initiate deleterious effects in rats when fed for about one-half their normal life span.

#### Publications:

ANDERSON, R. L., J. J. RACKIS, AND W. H. TALLENT. Biologically-Active Substances in Soy Products. Proc. Keystone Conference on Soybean Protein and Human Nutrition, Keystone, Colorado (in press).

CRAWFORD, C. G., R. D. PLATTNER, AND J. J. RACKIS. Separation of Oxidized and Unoxidized Phosphatidylcholine Molecular Species by High-Pressure Liquid Chromatography. Federation Proceedings (in press).

RACKIS, J. J., D. J. SESSA, AND D. H. HONIG. Flavor Problems of Vegetable Food Proteins. J. Am. Oil Chem. Soc. (in press).



RACKIS, J. J., J. E. MC GHEE, M. R. GUMBMANN, AND A. N. BOOTH. Effects of Soy Proteins Containing Trypsin Inhibitor in Long-Term Feeding Studies in Rats. J. Am. Oil Chem. Soc. (in press).

RACKIS, J. J. Conversion of Raw Soybeans Into High-Quality Protein Products. Proc. Symposium Soya and Soybean Protein for Human Nutrition, XI International Congress of Nutrition, Rio de Janeiro, Brazil (in press).

RACKIS, J. J. Soy Protein Foods. In Tropical Foods: Chemistry and Nutrition, Vol. 2, Academic Press, New York (in press).

RACKIS, J. J. AND M. R. GUMBMANN. Protease Inhibitors: Physiological Properties in Relation to Quality of Plant Proteins. In Antinutrients and Natural Toxicants in Foods, Symposium Series, American Chemical Society, Washington, D.C. (in press).

SESSA, D. J. AND R. D. PLATTNER. Novel Furaldehydes from Oxidized Soy Phospholipids. J. Agric. Food Chem. 27(1) (1979):209-210.

SESSA, D. J. Biochemical Aspects of Lipid-Derived Flavors in Legumes. J. Agric. Food Chem. (in press).

SESSA, D. J. Flavor Considerations of Soy Protein Products. Food Product Dev. (in press).

WOLF, W. J. AND D. J. SESSA. Lecithin. In Encyclopedia of Food Science, Edited by M. S. Peterson and A. H. Johnson, Avi Publishing Co., Inc., 1978, 461-467.

#### Other Reports:

HONIG, D. H. Effects of Residual Solvents and Storage on Flavor of Hexane:Ethanol Azeotrope Extracted Soy Products. Presented at National Meeting, American Chemical Society, Miami, Florida, September 1978.

HONIG, D. H. Enzymatic Determination of the Undigestible Residue Content (Dietary Fiber) of Soybean Products, Wheat, and Corn Bran. Presented at National Meeting of American Chemical Society, Miami, Florida, September 1978.

RACKIS, J. J. Soybean Goitrogenicity: A Review. Food Protein Council, Washington, D.C., May 1978.

RACKIS, J. J. Conversion of Raw Soybeans into High-Quality Protein Products. Presented at XI International Congress Nutrition, Rio de Janeiro, Brazil, August 27-September 1, 1978.

RACKIS, J. J. Flavor Problems of Vegetable Food Proteins. Presented at World Conference on Vegetable Food Proteins, Amsterdam, Netherlands, October 29-November 3, 1978.

RACKIS, J. J. Effects of Soy Proteins Containing Trypsin Inhibitors in Long-Term Feeding Studies in Rats. Presented at World Conference on Vegetable Food Proteins, Amsterdam, Netherlands, October 29-November 3, 1978.

RACKIS, J. J. Long-term Effects of Soybean Trypsin Inhibitors in Rats. Food Protein Council Meeting, Peoria, Illinois, October 13, 1978.

SESSA, D. J. Biochemical Aspects of Lipid-Derived Flavors in Legumes. Presented at ACS Meeting, March 1978, Anaheim, California.

SESSA, D. J. Flavor Considerations of Vegetable Proteins. Presented at Joint Symposium North Central Section, AOCS, Midwest Section of AACC and Chicago Section, Assoc. of Candy Technologists, March 1978, Chicago, Illinois.

SESSA, D. J. Current Concepts: Soy Protein Flavor. Presented to the Food Protein Scientific Committee. September 1978. Peoria, Illinois.

7. Interactions between Proteins and Oxidized Lipids of Soybeans  
(L. C. Wang, W. J. Wolf, and E. N. Frankel)

- a. Specific Objective: Develop methods to study soybean protein-oxidized lipid interactions.

Progress: A gas chromatographic method was developed to assay interactions between oxidation products of lipids and soybean proteins. With decane as an internal standard, the unreacted oxidized lipids were monitored in model systems with purified soy proteins. Azelaaldehyde esters, major lipid oxidation products, reacted as the methyl ester with the following purified soybean proteins: 2S, 7S, 11S, and polymerized 7S. The amount of azelaaldehyde bound to protein appeared proportional to protein concentration used in the range tested. These reactions may be significant with respect to the performance, safety, and nutritive value of soy proteins.

Publications:

WANG, L. C., J. F. CAVINS, AND W. J. WOLF. Gamma-glutamyl Dipeptide Content in Soybean Protein Products and Other Commodities. J. Food Sci. 43 (1978):740-745.

WANG, L. C. Ultrasonic Peptization of Soybean Proteins from Autoclaved Flakes and Alcohol Washed Flakes and Commercial Samples. J. Food Sci. 43 (1978):1311-1313.

8. Effect of Tocochromanol Dimers and Trimers on the Autoxidation of Fats  
(P.L. 480 Grant - Institute of Food Science, Poznan)

Using the analytical methodology already developed, the investigators have followed the decreasing content of alpha-, gamma-, and delta-



tocopherol and the increasing content of tocopherol dimers upon storage of crude and refined soybean oil as well as rapeseed, coconut, and palm oils, and certain Polish margarines. It was found that the amounts of dimers formed do not correspond to the amounts of tocopherols lost during storage. No explanation for the loss is available at the present time. Not all of the dimers formed by chemical oxidation of the tocopherols were found in the dimer products formed during storage. Cocoa shell fat was found to contain an unknown antioxidant in addition to three tocopherols. The unknown was superior to alpha-tocopherol as an antioxidant. Mixtures of beta-carotene and tocopherols were autoxidized to 20% destruction of the beta-carotene. The results indicated that beta-carotene had an activating effect on the formation of tocopherol dimers and trimers.

9. Influence of Triglyceride Structure on Formation of Geometrical Isomers on Soybean Oil (P.L. 480 Grant - Technical University, Gdansk)

Data were obtained on the effect of the triglyceride position of unsaturated fatty acids on the course of their hydrogenation. Over a wide range of temperature, pressure, stirring rate, and catalyst (type and concentration), the hydrogenation rate of each unsaturated fatty acid (octadecenoic, 18:1, octadecadienoic, 18:2, and octadecatrienoic, 18:3) was greater when the fatty acid moiety was located in the 1,3 positions, external, than in the 2 position, internal. Conversely, geometric isomerization proceeded at a higher rate at the internal positions than at the external positions. Several additional observations were made regarding the effect of reaction parameters on the course of hydrogenation. (1) Temperature: (a) as temperature increased trans-isomer selectivity increased; (b) the rate of hydrogenation increased sixfold as the temperature was raised from 140 C to 210 C. (2) Pressure: (a) doubling the reaction pressure from 1.5 atm to 3.5 atm gave a twofold increase in rate; (b) as pressure was increased trans-isomer formation decreased. (3) Stirring rate: (a) agitation had no effect on the rate of trans-isomer formation; (b) an increase in agitation produced an increase in hydrogenation rate. (4) Catalyst type and concentration: (a) regardless of catalyst type, selectivity was not influenced by concentration; (b) trans-isomer formation increased with concentration of unsupported catalyst; however, with supported catalyst, concentration had no effect; (c) influence on reaction rate depended on whether catalyst was supported or unsupported.

10. Nutritional and Physiological Studies of Soybean Hemagglutinins (P.L. 480 Grant - M.S. University of Baroda)

Ground rice in 1:2 combination with dal (coarsely ground meal) made from red kidney beans, black beans, or navy beans was made into a batter and fermented for 16 hr at 30 C after inoculation with Leuconostoc mesenteroides to yield idli, a fermented food. The hemagglutinin activities in the original legumes were completely inactivated as a result of the fermentation and therefore of no nutritional concern. Feeding of hemagglutinins from soybeans and



kidney beans to weanling rats caused increases of protease, hexosaminidase, and alpha-D-mannosidase activities in the intestine. Pretreatment of the hemagglutinins with L. mesenteroides abolished these responses in enzyme activity thereby supporting the conclusion that intact hemagglutinins rather than their hydrolysis products are responsible for elevating enzyme activities in the intestine. The ability of extracts of L. mesenteroides to inactivate soybean hemagglutinin is attributed to three enzymes: protease, beta-N-acetyl glucosaminidase and alpha-D-mannosidase, but the agglutination activity is lost only when the hemagglutinin is treated with alpha-D-mannosidase. These results suggest that the mannose moiety must be intact for the agglutinin to bind to the receptor site in red blood cells to cause agglutination.

### C. TECHNOLOGIES FOR INDUSTRIAL USES - PLANT AND ANIMAL PRODUCTS

#### 1. Chemical Modification of Soybean Oil and Its Derivatives (E. N. Frankel)

- a. Specific Objective: Prepare energy-sparing water-dispersible coatings from renewable resources.

Progress: The stability and film properties of water-dispersible anionic poly(ester-amide-urethane) resins made from soybean oil were significantly improved by selecting specific anhydride components that are not readily hydrolyzed in the resin. Thus, a commercial polyanhydride (1:1 octadecene-1 and maleic anhydride copolymer) produced dispersions that were stable for more than 13 months.

Cationic urethane polyesteramides suitable for emulsion systems have also been developed. They show promise as more environmentally desirable coatings because they contain only water and no volatile organic solvents. Films from an emulsion of a soybean oil-isophthalic-tolylene diisocyanate modified resin yield hard and rapid drying coatings. Initial water resistance is better than for the dispersed resins. By a modified synthesis, either cationic or anionic systems are possible.

- b. Specific Objective: Prepare organosulfur compounds for evaluation as sulfurized sperm oil replacements in lubricant additives.

Progress: Octadecyl sulfide, disulfide, trisulfide and tetrasulfide, and tetrasulfides from 9(10)-mercaptostearic acid were prepared and characterized by mass, nuclear magnetic resonance, and Raman spectroscopy, as model compounds in our basic studies on the physico-chemical properties of these compounds. Chemical structure has a marked effect on extreme pressure properties. For the octadecyl series the following order of effectiveness was noted: Tetrasulfide > trisulfide > disulfide > sulfide. Industrial evaluation confirmed our findings that liquid crude tetrasulfides (14.1% S) were superior to crystalline high purity octadecyl tetrasulfide (19.3% S). Both samples were also

superior to a commercial sulfurized oleic acid (10% S), an additive currently used, but slightly inferior to low-cost sulfurized isobutylene (45.5% S). However, this commercial additive has poor thermal stability.

- c. Specific Objective: Prepare new derivatives of geminal bis(hydroxymethyl) fatty esters and silicon-containing compounds as substitutes or complements for petroleum-derived materials.

Progress: In our basic studies on new derivatives made from unsaturated fatty materials and low-cost silicon compounds, dichloromethylsilane was added to methyl oleate by refluxing with chloroplatinic acid catalyst. A method for gas-liquid chromatographic analysis was developed and showed the presence of at least three major components. The product is thermally stable and not highly reactive with atmospheric moisture. Attempts to prepare a simpler, chlorine-free silicon derivative by catalytic hydrosilylation of methyl oleate with triethylsilane, trimethoxysilane, and triethoxysilane, have been so far unsuccessful. No work was carried out on geminal bis(hydroxymethyl) fatty esters in order to concentrate our efforts on the silicon derivatives.

Publications:

ADAMS,\* C., E. N. FRANKEL, AND J. H. P. UTLEY.\* The Anodic Acetoxylation of Methyl Oleate (\*Queen Mary College, University of London). J. Chem. Soc. (Perkin Transactions II), in press.

BELL, E. W. Wax Esters of Vegetable Oil Fatty Acids Useful as Lubricants. U.S. Patent Allowed.

DUFEK, E. J. Conversion of Methyl 9(10)-Formylstearate to Carboxymethylstearate. J. Am. Oil Chem. Soc. 55 (1978):337-339.

FRANKEL, E. N. AND E. H. PRYDE. Acetoxymethyl Derivatives of Polyunsaturated Fatty Triglycerides as Primary Plasticizers for Polyvinylchloride. U.S. Patent 4,083,816 (April 11, 1978).

FRANKEL, E. N. Homogeneously Catalyzed Reactions. In "Fatty Acids," edited by E. H. Pryde, American Oil Chemists' Society, Champaign, Illinois, in press, chapter 22.

FRIEDRICH, J. P. Lubricants. In "Fatty Acids," edited by E. H. Pryde, American Oil Chemists' Society, Champaign, Illinois, in press, chapter 30.

JOHNSON, R. W. AND E. H. PRYDE. Isomerization, Conjugation, and Cyclization. In "Fatty Acids," edited by E. H. Pryde, American Oil Chemists' Society, Champaign, Illinois, in press, chapter 16.

MILLER, W. R., E. H. PRYDE, AND G. R. RISER. 9,9(10,10)-Bis(acetoxymethyl)-Octadecanoate Esters as Plasticizers for Poly(vinyl chloride). J. Am. Oil Chem. Soc. 55 (1978):469-470.

MILLER, W. R. AND E. H. PRYDE. Alkyl 9,9(10,10)-Bis(acyloxymethyl)-octadecanoates as Primary Plasticizers for Polyvinylchloride. U.S. Patent 4,093,637 (June 6, 1978).

MILLER, W. R. AND E. H. PRYDE. Alkyl 9,9(10,10)-Bis(acyloxymethyl)-octadecanoates as Primary Plasticizers for Polyvinylchloride. U.S. Patent 4,124,558 (November 7, 1978).

PRYDE, E. H. Carboxylic Acids--Survey. Kirk-Othmer: Encycl. Chem. Technol. M. Grayson, exec. ed., Vol. 4, 3rd edition, John Wiley & Sons, Inc., New York, 1978, pp. 814-834.

PRYDE, E. H. Carboxylic Acids--Economic Aspects. Kirk-Othmer: Encycl. Chem. Technol. M. Grayson, exec. ed., Vol. 4, 3rd edition, John Wiley & Sons, Inc., New York, 1978, pp. 853-859.

PRYDE, E. H. Natural Fatty Acids and Their Sources. In "Fatty Acids," edited by E. H. Pryde, American Oil Chemists' Society, Champaign, Illinois, in press, chapter.

PRYDE, E. H. Electrochemical Reactions. In "Fatty Acids," edited by E. H. Pryde, American Oil Chemists' Society, Champaign, Illinois, in press, chapter 24.

RAKOFF, H. AND L. E. GAST. Optical Brighteners--Their Effect on the Yellow Appearance of Linseed Oil Paint. J. Coatings Technol. 50 (1978):84-85.

RAKOFF, H., F. L. THOMAS, AND L. E. GAST. Reversibility of the Yellowing Phenomenon in Linseed Based Paints. J. Coatings Technol., in press.

SCHNEIDER, W. J. AND L. E. GAST. Water Dispersible Urethane Polyester-amide Coatings from Linseed Oil. J. Coatings Technol. 50 (1978):76-81.

SCHNEIDER, W. J. AND L. E. GAST. Triglyceride Oil-Derived Water Dispersible Urethane Resin Coatings. U.S. Patent 4,094,838 (June 13, 1978).

SCHWAB, A. W., W. K. ROHWEDDER, AND L. E. GAST. Hydrogen Sulfide Adducts of Methyl trans,trans-9,11-Octadecadienoate. J. Am. Oil Chem. Soc. 55 (1978):860-864.

#### Other Reports:

SCHNEIDER, W. J. Poly(ester-amide-urethane) Water Dispersible and Emulsifiable Resins. Presented at Federation of Societies for Coatings Technology, November 1978.

SCHWAB, A. W. Mass, Nuclear Magnetic Resonance and Raman Spectroscopy of Octadecyl Sulfide, Disulfide and Tetrasulfide. Presented at the



#### D. FOOD COMPOSITION AND IMPROVEMENT

##### 1. Composition and Properties of Cereal Grain Fibers for Foods (E. W. Bell)

See Cereal Science and Foods Laboratory, C.1.

#### E. HUMAN REQUIREMENTS FOR NUTRIENTS

##### 1. Preparation of Labeled Isomeric Fats for Human Ingestion and Analysis of Their Metabolic Products (E. A. Emken)

- a. Specific Objective: Continue studies to compare utilization in humans of cis-12- and trans-12-octadecenoic acids to cis-9-octadecenoic acid. This work will provide basic information on the nutritional impact of isomeric fats formed during hydrogenation of soybean oil.

Progress: A mixture containing triglycerides of trans-12-octadecenoate-9,10-d<sub>2</sub> (9.88 g), cis-12-octadecenoate-15,15,16,16-d<sub>4</sub> (9.88 g) and cis-9-octadecenoate-14,14,15,15,17,18-d<sub>6</sub> (9.0 g) was fed to a 26-year-old male subject. Personnel from St. Francis Hospital-Medical Center, Peoria, drew blood samples which were separated into plasma, red blood cell, platelet, and lipoprotein fractions. These fractions were extracted and the blood lipids separated into individual sub-fractions which were then derivatized and analyzed by mass spectroscopy. Chylomicron data show the cis- and trans-12- and cis-9-octadecenoates are absorbed equally well. The cis-12-octadecenoate was incorporated into various phospholipid fractions to a greater extent than the trans-12- or cis-9-octadecenoic acids. The selection for cis-12-octadecenoate was very large for both the 1-acyl and 2-acyl position of phosphatidylcholine indicating the cis-12-octadecenoic acid isomer may be competing with incorporation of linoleic acid which is normally the predominant fatty acid in the 2-acyl position of this phospholipid. Selective incorporation of trans-12-octadecenoate into phosphatidylcholine was also noted but trans-12-octadecenoate was concentrated in the 1-acyl position suggesting that it is competing with stearic acid rather than linoleic acid. An almost absolute exclusion against incorporation of trans-12-octadecenoate into 2-acyl phosphatidylcholine occurred. Similarly, incorporation of trans-12-octadecenoate into cholesteryl ester was also very low. Complete turnover of these fatty acids occurred within 48 hours.

- b. Specific Objective: Develop practical synthetic methods for preparation of deuterated octadecadienoic acids.

Progress: Methods were developed for the preparation of various key intermediates needed for the synthesis of deuterated octadecadienoic acids. 3-Nonynol-8,8,9,9,-d<sub>4</sub> was prepared by acetylenic coupling of

3-butyn-1-ol with iodopentane-4,4,5,5-d<sub>4</sub>. Various reaction conditions were investigated to determine the feasibility of preparing 12,15-octadecadienoic acids via the Wittig reaction. Methods for reducing enynes and diynes to all cis or all trans dienes were developed; lithium in ammonia-methanol yielded all trans dienes, and palladium on barium sulfate yielded all cis dienes. Procedures were established for conversion of octadecadienoic acids to triglycerides without accompanying isomerization. These developments now assure that deuterated octadecadienoic acid isomers can be synthesized on a practical basis. New analytical and separation techniques, notably silver resin chromatography, were developed to aid in the preparation and identification of intermediates necessary for synthesis of deuterated dienes.

- c. Specific Objective: Achieve operational status with Finnigan mass spectrometer to increase numbers of samples analyzed and to increase sensitivity and accuracy of analysis.

Progress: Electronic equipment for controlling mass spectrometry operations has been built and computer programming necessary for routine mass spectrometric analysis of samples containing mixtures of deuterium-labeled fatty acids has been completed. These developments decrease the time required to analyze samples and allow a wide range of unknown sample sizes to be utilized without loss of sensitivity or accuracy.

Publications:

ADLOF, R. O., W. R. MILLER, AND E. A. EMKEN. Synthesis of cis and trans Methyl 8- and 13-Octadecenoate-d<sub>2</sub> and d<sub>4</sub> Isomers. J. Labelled Compd. Radiopharm. 15 (1978): 625-636.

ADLOF, R. O. AND E. A. EMKEN. Synthesis of Methyl cis-9-Octadecenoate-14,14,15,15,17,18-d<sub>6</sub>. J. Labelled Compd. Radiopharm. 15 (1978):97-104.

DEJARLAIS, W. J. AND E. A. EMKEN. Synthesis of Di-, Tetra-, and Hexadeuterated 11-Octadecenoates. J. Labelled Compd. Radiopharm. 15 (1978):451-460.

EMKEN, E. A. Commercial and Potential Utilization of Lipoxxygenase. J. Am. Oil Chem. Soc. 55 (1978):416-421.

EMKEN, E. A. Effects of Fatty Acid Isomer in Humans. In Geometrical and Positional Isomeric Fatty Acids, Chapter 5, Edited by E. A. Emken and H. J. Dutton, American Oil Chemists' Society Publishers, 1979 (in press).

EMKEN, E. A., W. K. ROHWEDDER, H. J. DUTTON, W. J. DEJARLAIS, R. O. ADLOF, J. MACKIN, AND J. M. IACONO. Incorporation and Distribution of Deuterium Labeled Elaidic Acid in Man: Neutral Blood Lipids. Metabolism (in press).

EMKEN, E. A., W. K. ROHWEDDER, H. J. DUTTON, W. J. DEJARLAIS, R. O. ADLOF, J. F. MACKIN, R. DOUGHERTY, AND J. M. IACONO. Incorporation of Deuterium Labeled cis and trans-9-Octadecenoic Acids in Humans: Plasma, Erythrocyte and Platelet Phospholipids. J. Am. Oil Chem. Soc. 55 (1978): 259a.

EMKEN, E. A. Nutritional Value of Soybean Oil. In American Soybean Association Soybean Oil Handbook, Edited by O. L. Brekke, T. L. Mounts, and E. H. Pryde, 1979 (in press).

EMKEN, E. A., J. C. HARTMAN, AND C. R. TURNER. Separation of Octadecadienoate, Octadecatrienoate and Acetylenic Fatty Acids by Silver Resin in Chromatography. J. Am. Oil Chem. Soc. 55 (1978):561-563.

EMKEN, E. A. Synthesis and Analysis of Stable and Radioisotope-Labeled Fatty Acids. In Handbook of Lipid Research, Vol. 1, Chapter 2, Edited by Arnis Kuksis, 1978, pp. 77-121.

EMKEN, E. A., R. O. ADLOF, W. J. DEJARLAIS, AND H. RAKOFF. Synthesis of Deuterated Octadecenoic Acid Isomers for Human Studies. Proceedings of the Third International Conference on Stable Isotopes (in press).

GAFFNEY, J., A. IRSA, L. FRIEDMAN, AND E. A. EMKEN.  $^{13}\text{C}/^{12}\text{C}$  Analysis of Vegetable Oils, Starches, Proteins and Soy-Meat Mixtures. J. Agric. Food Chem. Accepted January 1979.

RAKOFF, HENRY, DAVID WEISLEDER, AND EDWARD A. EMKEN.  $^{13}\text{C}$  Nuclear Magnetic Resonance of Mono and Dihydroxy Saturated and Unsaturated Fatty Methyl Esters. Lipids 14(1979):81-83.

RAKOFF, H. AND E. A. EMKEN. Silver-Resin Chromatographic Separation of cis and trans- Mono- and Dihydroxy Fatty Esters. J. Am. Oil Chem. Soc. 55 (1978):564-566.

RAKOFF, HENRY, R. O. ADLOF, AND E. A. EMKEN. Synthesis of cis and trans Methyl 12-Oxo-9-dodecenoates, Their Corresponding Acetals and Methyl 12-Oxo-trans-10-dodecenoate. Synth. Commun. (in press).

RAKOFF, H. AND E. A. EMKEN. Synthesis of Geometric Isomers of Di-, Tetra-, and Hexadeuterated 12-Octadecenoates and their Triglycerides. J. Labelled Compd. Radiopharm. 15 (1978):233-252.

#### Other Reports:

EMKEN, E. A. Incorporation of Deuterium Labeled cis- and trans-9-octadecenoic Acids in Humans: Plasma, Erythrocyte and Platelet Phospholipids. Presented at American Oil Chemists' Society Meeting, St. Louis, Missouri, May 14-18, 1978.

EMKEN, E. A. Incorporation of Isomeric Fatty Acids in Human Blood and Egg Lipids. Presented at the Conference of General Collaborators, Peoria, Illinois, October 23-24, 1978.



EMKEN, E. A. Separation of Saturated, Unsaturated and Acetylenic Fatty Acid Isomers by Silver Resin Chromatography. Presented at American Oil Chemists' Society Meeting, St. Louis, Missouri, May 14-18, 1978.

EMKEN, E. A. Synthesis of Deuterated Octadecenoic Acid Positional and Geometric Isomers. Presented at Third International Conference on Stable Isotopes, Oakbrook, Illinois, May 23-26, 1978.

RAKOFF, HENRY. Silver Resin Chromatographic Separation of cis and trans Mono- and Dihydroxy Fatty Esters. Presented at American Oil Chemists' Society Meeting, St. Louis, Missouri, May 14-18, 1978.

RAKOFF, HENRY. Synthesis of Methyl 12-Oxo-cis and trans-9-dodecenoates and their Conjugated Analog. Presented at American Oil Chemists' Society Meeting, St. Louis, Missouri, May 14-18, 1978.

ANONYMOUS. Dietary Fats Traced Without Radioisotopes. Chem. Eng. News, May 29 (1978):page 19.

ANONYMOUS. Where Does All the Fat Go? Chemistry 51 (1978):21.

Reynolds, Geri and Lane, Dave. Research Here May Ease Risks of Heart Attacks, Other Diseases. Peoria Journal Star, May 12, 1978.

2. Biological Utilization of Isomeric Fatty Acids from Hydrogenated Soybean Oil (E. A. Emken)

- a. Specific Objective: Prepare tritium-labeled trans-8, 10, 11, and 12 isomers of oleic acid for use in laying hen experiments.

Progress: trans Positional fatty acid isomers are found in partially hydrogenated vegetable oils but their nutritional value and metabolic fate are unknown. Tritiated methyl trans-12-octadecenoate was prepared by toluenesulfinic acid isomerization of tritiated cis-12-octadecenoate. Reaction products were purified by silver nitrate-silica gel thin-layer chromatography. Availability of this radioisotope labeled fatty acid will permit its utilization in biological systems to be evaluated.

- b. Specific Objective: Initiate feeding studies with laying hen using trans positional isomers of oleic acid.

Progress: Feeding studies with the laying hen using tritiated trans-12-octadecenoate and carbon-14 labeled oleate were completed. Individual egg neutral lipids and phospholipids were separated and analyzed. Incorporation of trans-12-octadecenoic acid vs oleic acid was measured. trans-12-Octadecenoic acid was preferentially utilized by the phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, and the cholesteryl ester fractions. These studies provide basic information on the biochemical fate of one of the trans positional isomers found in partially hydrogenated vegetable oils.

- c. Specific Objective: Positional acyl analysis of phospholipids from linoleate vs linoelaidate studies in the laying hen.

Progress: Acyl positional analysis for egg phosphatidyl ethanolamine (PE) and egg phosphatidylcholine (PC) were completed. Results from studies using tritium and carbon-14 labeled linoelaidate and linoleate indicate linoelaidate was preferentially incorporated into the 1-acyl position and linoleate was selectively utilized in the 2-acyl position of PE and PC. Tritium/ carbon-14 ratios from a linoleate control experiment detected an isotope effect during linoleate incorporation into the 1-acyl position of PE and PC. This isotope effect appears to be due to tritium at the 12-double bond position. These results supply basic information for predicting the nutritional consequence of dietary linoleic acid isomers in partially hydrogenated vegetable oil and for evaluating the validity of radioisotope tracer experiments in the laying hen.

Publications:

LANSER, A. C. Metabolism of Isomeric Fats in the Laying Hen. In Geometrical and Positional Isomeric Fatty Acids, Chapter 4, Edited by E. A. Emken and H. J. Dutton, American Oil Chemists' Society Publishers, 1979 (in press).







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